



Impact of Biomarker Heterogeneity on Pediatric Migraine Pathophysiology and Treatment

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Abstract

Pediatric migraine affects roughly 10% of children and adolescents worldwide, representing a significant cause of reduced quality of life. Current diagnosis according to the International Classification of Headache Disorders, 3rd Edition (ICHD-3) is based on clinical symptoms. This approach may not entirely capture the physiology underlying migraine phenotype, severity, frequency, and treatment response due to the heterogeneity of biomarkers. Biomarkers may therefore complement clinical assessment by providing objective measures of biological processes underlying pediatric migraine. This literature review evaluates the current evidence for biomarkers associated with the pathophysiology and clinical characteristics of pediatric migraine and assesses their potential utility in diagnosis and patient characterization. PubMed and Google Scholar were searched for primary research studies and review articles examining biomarkers associated with pediatric migraine pathophysiology, clinical presentation, diagnosis, and treatment; the literature review emphasized studies published within the past ten years. The literature tracked the following biomarker classes: neuropeptides, inflammatory biomarkers, metabolic and mitochondrial biomarkers, oxidative-stress biomarkers, vitamin-related biomarkers, microRNAs, and vascular biomarkers. These findings identify several potential biomarker candidates (e.g. CGRP, inflammatory biomarkers) that may complement clinical assessment and, when evaluated in combination, may contribute to a multimarker diagnostic tool for pediatric migraine. Future research should prioritize larger longitudinal pediatric studies and standardized biomarker measurements to better translate biomarker profiles to clinically useful diagnostic and treatment strategies.

Key Words: pediatric migraines, biomarker heterogeneity, migraine pathophysiology, diagnostics, personalized care

1. Introduction

1.1 Overview of Migraine Disorders

Migraine is a primary headache disorder arising from intrinsic neurological mechanisms rather than secondary structural abnormalities (1, 2). A complex neurological disorder, it is characterized by recurrent episodes of altered sensory processing, abnormal neuronal excitability, and dysregulated neurovascular signaling (1, 3). Approximately one-third of patients experience migraine with aura, a transient neurological syndrome characterized by reversible visual, sensory, language, motor, or brainstem symptoms that typically precede or accompany the headache phase (3). Aura refers to fully reversible neurological symptoms associated with changes in cortical function, blood circulation, and neurovascular integration; among the different varieties, visual auras are the most frequent and may include positive symptoms, such as bright lines or shapes, or negative symptoms, such as reduced or lost vision (1). Although a moderate-to-severe headache is the most recognizable feature of migraine, the disorder affects a variety of bodily processes. It involves dysfunction across interconnecting central and nervous system pathways responsible for pain transmission, sensory integration, and autonomic regulation (2).

Migraine attacks typically last 4-72 hours in adults and are commonly described as unilateral (one-sided), pulsating, and aggravated by routine physical activity, although it varies among individuals. Attacks are frequently accompanied by nausea, allodynia (extreme sensitivity to touch), vomiting, photophobia, osmophobia, and phonophobia, demonstrating the involvement of sensory and autonomic pathways beyond nociceptive signaling alone (4). Understanding migraine as a disorder involving interconnected sensory, neuronal, and autonomic pathways provides a foundation for examining how its mechanisms affect different patient populations. Figure 1 as depicted below further explores the locations of these symptoms.

Figure 1. Common sensory, neurological, and autonomic symptoms associated with migraine patients are depicted in the figure above, illustrating the range of clinical manifestations that may occur during a migraine attack.

1.2 Pathophysiology and Classification of Migraines in the General Population

Key brain regions and pathways involved in migraine include the cerebral cortex, brainstem, thalamus, hypothalamus, and trigeminal nerve system. The cerebral cortex is involved in sensory processing, the brainstem and hypothalamus are involved in regulating sensory processing and autonomic functions, and the thalamus serves as an important relay center for sensory and pain-related information. The trigeminal nerve system provides sensory innervation to pain-sensitive intracranial structures, including the meninges and associated blood vessels (1, 2).

The clinical manifestations of migraine arise from interactions among several interconnected biological mechanisms, including trigeminovascular activation, neurogenic inflammation, cortical spreading depression (CSD), and central sensitization. The trigeminovascular system plays a central role in migraine initiation and consists of trigeminal sensory neurons that innervate intracranial structures, like the meninges and associated blood vessels. Activation of these pathways stimulates the release of vasoactive neuropeptides, including calcitonin gene-related peptide (CGRP), which promotes nociceptive signaling and contributes to headache development (5).

Following trigeminovascular activation, neurogenic inflammation occurs through the release of inflammatory mediators that increase the meningeal nociceptor's sensitivity and amplify pain transmission (5). Cortical spreading depression (CSD) is considered the physiological basis of migraine aura, and may activate trigeminovascular pathways, linking these neurological symptoms with headache generation (6). Repeated activation of these migraine-associated pathways can also lead to central sensitization, where nociceptive neurons become increasingly responsive to sensory input. This mechanism contributes to common symptoms like allodynia (heightened pain intensity, and increased sensitivity to environmental stimuli like light and sound (i.e. phonophobia and photophobia) (2).

Currently, the International Classification of Headache Disorders, Third Edition (ICHD-3) developed by the International Headache Society serves as the internationally accepted diagnostic framework for migraine and other headache disorders. It classifies migraine based on a patient's medical history and symptom presentation rather than objective laboratory or imaging findings. Diagnosis relies on evaluation of headache characteristics, attack frequency and

duration, associated symptoms, and the presence and absence of aura (7). Using these standardized criteria, the ICHD-3 further distinguishes migraine subtypes, as summarized in Table 1: migraine without aura, migraine with aura, chronic migraine, probable migraine, and episodic syndromes associated with migraine; these criteria have been simplified over time for the ease of use across multiple contexts (8). Although the ICHD-3 provides a standardized framework for classifying migraine based on clinical characteristics, these diagnostic categories do not entirely capture the developmental factors that may influence migraine pathophysiology.

Table 1. ICHD-3 migraine diagnoses and related episodic syndromes are depicted below. Diagnostic categories and clinical characteristics are summarized from the International Classification of Headache Disorders, 3rd Edition (ICHD-3). Diagnostic criteria should be interpreted according to the complete ICHD-3 criteria rather than the abbreviated descriptions provided in this table.

ICHD-3 Diagnosis	Key Clinical Characteristics
Migraine without aura	Recurrent attacks of headache lasting 4-72 hours; typically unilateral location, pulsating, moderate to severe intensity, aggravated by physical activity, and associated nausea/vomiting or photophobia and phonophobia symptoms
Migraine with aura	Recurrent attacks lasting minutes of fully reversible visual, sensory, speech/language, motor, brainstem, or retinal aura symptoms before or during the headache
Chronic migraine	Headache occurring 15+ days per month for more than 3 months, or at least 8 days per month, with the feature of migraine headache
Probable migraine	Migraine-like attacks that fulfill all but one of the diagnostic criteria for a specific migraine diagnosis and not better accounted for by another ICHD-3 diagnosis
Episodic syndromes associated with migraine	Recurrent episodic disorders that occur predominantly in children and adolescents and are associated with migraine (e.g. cyclic vomiting syndrome, abdominal migraine, benign paroxysmal vertigo)

1.3 An Introduction to Pediatric Migraines

While the clinical focus often centers on the adult population, migraine also poses a major burden for the pediatric population, affecting up to 10% of youths and causing significant functional impairment (9). Although pediatric migraine shares many underlying mechanisms with adult migraine, its clinical presentation differs due to ongoing neurological development. Throughout childhood and adolescence, maturation of cortical networks, neurotransmitter systems, pain-processing pathways, and autonomic regulation may influence migraine

susceptibility and symptom presentation. Therefore, pediatric migraine should not be considered as an earlier manifestation of adult disease, as developmental processes can alter the clinical phenotype and biological mechanisms underlying migraine (10).

These differences are reflected in pediatric migraine presentations. The ICHD-3 recognizes that migraine attacks in children and adolescents may last 2-72 hours due to ongoing nervous system maturation and differences in brain development, in comparison to the typical 4-72 hour duration seen in adults. Pediatric migraine is also more commonly bilateral, particularly in the frontotemporal region, whereas adult migraine is typically unilateral. Additionally, younger children may have difficulty identifying and communicating subjective sensory symptoms, requiring clinicians to interpret behavioral indicators like phonophobia and photophobia. Pediatric patients may also experience migraine-associated episodic syndromes, including abdominal migraine, gastroparesis, and cyclic vomiting syndrome (11).

This reliance on clinical phenotype presents several limitations in pediatric migraine. Symptoms such as headache severity, sensory sensitivity, and associated neurological experiences are inherently subjective; they may be difficult for pediatric patients to accurately describe due to developmental differences in language, cognition, and pain perception. Furthermore, its rigid metric offers limited flexibility to patients whose symptoms deviate from typical presentation (e.g. children with shorter attacks or patients with bilateral headache) (12).

1.4 Purpose of this Study

Objective biological markers, rather than replacing clinical classification, may provide complementary information by identifying biological processes underlying observable symptom patterns (13). Unlike the subjective clinical diagnostic criteria, biomarkers provide molecular- and cellular-level insight into the biological mechanisms underlying disease. Recent migraine research has identified several biomarker classes associated with migraine pathophysiology, including neuropeptides, inflammatory cytokines, mitochondrial biomarkers, vascular biomarkers, circulating metabolites, and microRNAs (13).

Given the molecular heterogeneity of pediatric migraine and the developmental factors that influence biomarker expression, understanding how these molecular signatures may provide insight beyond current symptom-based diagnostic frameworks. A recent systematic review by Monte et al., biochemical biomarkers synthesized existing evidence on biochemical biomarkers in pediatric migraine (14). The current literature review expands the scope of biomarker investigation to include nutritional and vascular biomarkers and further examines the relationship between biomarker trends, clinical presentation, and treatments.

Accordingly, this review addresses the following research question: How are variations in biomarker classes associated with differences in the mechanism of pediatric chronic and episodic migraines' onsets, and can they inform corresponding treatments?

2. Methods

A literature review was conducted using PubMed and Google Scholar to identify primary research studies and review articles investigating biomarkers associated with pediatric migraine pathophysiology, diagnosis, symptom presentation, and current and emerging pharmacologic

treatments. Searches were performed using combinations of keywords related to pediatric migraine, biomarker pathways, diagnosis, symptoms, and treatment.

Table 2. Search categories and terms used to identify literature on biomarkers, clinical characteristics, and pharmacologic treatment of pediatric migraine. Search terms were combined using Boolean operators (AND/OR) to identify literature related to pediatric migraine biomarkers, clinical presentation, diagnosis, pathophysiology, and pharmacologic treatment.

Review Topic	Search category	Search terms
Core phrases	Population and condition	“migraine” OR “pediatric” OR “child” OR “children” OR “adolescent”
	Biomarkers and pathophysiology	“trigeminal” OR “migraine pathophysiology” OR “biomarker” OR “peptide”
Diagnostics and Biomarker Trends	Neuropeptides	“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 “neurogenic inflammation”
	Inflammatory/immune	“C-reactive protein” OR “CRP” OR “pentraxin-3” OR “IL-1 β ” OR “Interleukin-1 β ” OR “Interleukin-6” OR “IL-6” OR “tumor necrosis factor- α ” OR “TNF- α ” OR “PTX-3” OR “cytokines” OR “immunoglobulin G” OR “immunoglobulin A”
	Oxidative stress/mitochondrial	“growth differentiation factor” OR “GDF-15” OR “fibroblast growth factor” OR “FGF-21” OR “hypoxia-inducible factor-1 alpha” OR HIF-1 α
	Metabolic biomarkers	“tryptophan” OR “serotonin” OR “5-hydroxytryptamine” OR “5-HT” OR “kynurenine” OR “kynurenic acid”
	Nutritional biomarkers	“vitamin” OR “vitamin B2” OR “riboflavin” OR “coenzyme Q10” OR “CoQ10”

	Epigenetic regulators	"microRNA" or "miRNA"
	Vascular biomarkers	"Endothelin-1" OR "ET-1" OR "pentraxin-3" OR "PTX-3" OR "vascular endothelial growth factor" OR "VEGF"
	Diagnostic applications	"plasma levels" OR "assays" OR "biological panels"
Clinical presentation	Clinical symptoms	"aura" OR "photophobia" OR "phonophobia" OR "allodynia" OR "nausea" OR "vomiting" OR "prodrome" OR "focal pain" OR "mood changes" OR "speech difficulty" OR "visual disturbances" OR "dizziness" OR "vertigo" OR "lightheadedness"
	Physiological mechanisms	"trigemino-vascular" OR "trigemino-vascular activation" OR "neurogenic inflammation" OR "central sensitization" OR "cortical spreading depression" OR "CSD"
Pharmacologic treatment	Treatment-specific	"pharmacology" OR "pharmacologic" OR "therapy" OR "therapeutics" OR "management" OR "intervention" OR "preventive" OR "abortive"
	Therapeutics classes	"calcitonin gene-related peptide monoclonal antibodies" OR "calcitonin gene-related peptide mAbs" OR "CGRP monoclonal antibodies" OR "CGRP mAbs" OR "gepants" OR "CGRP receptor antagonists" OR "selective serotonin agonists" OR "tricyclic antidepressants" OR "selective serotonin reuptake inhibitor" OR "SSRI" OR "serotonin-norepinephrine reuptake inhibitor" OR "SNRI" OR "monoamine oxidase inhibitor" or "MAOI"

Studies published within the past ten years were preferred to ensure the inclusion of current evidence. Both primary research articles and high-quality review articles were included. Older studies were incorporated when they represented landmark findings or provided key evidence essential for understanding biomarker mechanisms.

A recent systematic review conducted by Monte et. al identified similar trends in biomarker expression across both pediatric and adult populations (14). The study found expression of

certain biomarker classes, namely neuropeptides, inflammatory biomarkers, oxidative stress markers, gut microbiota-derived metabolites, and microRNAs, in pediatric patients appear to be developmentally modulated by age-related physiological differences and disease duration. Accordingly, the following review incorporates adult studies when pediatric-specific evidence is limited, using these adult findings to provide complementary mechanistic and biological context rather than serving as a direct substitute for pediatric-specific evidence.

2.1 Biomarker Heterogeneity in Pediatric Migraine

Pediatric migraine is a biologically heterogeneous disorder. Although the trigeminovascular system remains central to contemporary models of migraine pathophysiology (9), accumulating evidence suggests that neuropeptide signaling, immune activation, metabolic dysregulation, mitochondrial dysfunction, vascular alterations, and epigenetic regulation collectively influence disease susceptibility and progression. The relative contribution of these pathways likely varies among individuals, providing a potential basis for the marked heterogeneity observed in clinical presentation and therapeutic response (14).

2.1.1 Biomarker Classes in Migraines

This literature review covers the following biomarker classes; this classification is not intended to be exhaustive and instead highlights the principal biomarker pathways currently investigated in pediatric migraine.

Table 3. Biomarker classes, proposed roles in pediatric migraines, and representative biomarkers are depicted below. Biomarkers are categorized according to their predominant biological pathways and potential roles in pediatric migraine pathophysiology and clinical presentation. The proposed roles represent mechanisms described in the literature and do not indicate that any biomarker has been validated as a diagnostic or prognostic marker. Biomarker classes may overlap because individual biomarkers can participate in multiple biomarker pathways.

Biomarker Class	Description	Proposed Role in Migraine	Examples
Neuropeptides (15)	Modulate synaptic transmission and influence physiological processes	Pain signaling, vascular regeneration, trigeminovascular action, and neurogenic inflammation	Calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38), vasoactive intestinal peptide (VIP), substance P (SP), and

neuropeptide Y
(NPY)

Inflammatory Biomarkers (16)	Activate innate and adaptive immune response	Peripheral and central sensitization	Cytokines, C-reactive protein (CRP), pentraxin-3 (PTX-3), interleukins
Metabolic biomarkers (17)	Offers insight into diagnosis, prognosis, and treatment response to diseases based on a specific metabolic signature	Indicate alterations in biochemical pathways that regulate neuronal excitability, neurotransmitter synthesis, and cellular homeostasis	Tryptophan, serotonin (5-hydroxytryptamine; 5-HT), kynurenine, kynurenic acid
Mitochondrial function and oxidative stress biomarkers (18)	Indicate alterations in cellular energy metabolism and oxidative balance	Cortical spreading depression and trigeminovascular activation	Growth differentiation factor-15 (GDF-15), fibroblast growth factor-21 (FGF-21), and hypoxia-inducible factor-1 alpha (HIF-1 α).
Vitamin-related biomarkers (19)	Deficiencies of such micronutrients may contribute to reduced cellular energy availability and increased oxidative stress	Indicate metabolic and mitochondrial processes involved in migraine susceptibility	Riboflavin (Vitamin B2), coenzyme Q10 (CoQ10),
MicroRNAs (20)	Regulate gene expression in inflammatory and anti-inflammatory signaling pathways	Affect trigeminovascular activation, central sensitization, and migraine susceptibility	hsa-miR-34a-5p, hsa-miR-375

Vascular biomarkers (21)	Provide insight into endothelial function, vascular regulation, and cerebrovascular responses	Influences migraine susceptibility and symptom expression through interaction with the trigeminovascular system	Endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and pentraxin-3 (PTX-3)
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3. Mechanistic Contributions of Biomarker Pathways to Pediatric Migraine Pathophysiology

The biomarker pathways implicated in pediatric migraine represent interconnected biological processes rather than isolated mechanisms. Alterations across these pathways may collectively influence migraine susceptibility and progression through effects on trigeminovascular activation, neurogenic inflammation, neuronal excitability, energy metabolism, vascular regulation, and epigenetic control of gene expression. The following sections examine evidence supporting each biomarker class and evaluate its proposed mechanistic contribution to pediatric migraine pathophysiology. Figure 2 further explains the most common location of the following biomarker classes discussed.

Figure 2. The figure illustrates the distribution of biomarker classes implicated in pediatric migraine pathophysiology and their associated anatomical and biological location. The distribution of biomarkers involved in interconnected processes, including trigeminovascular activation, neurogenic inflammation, neuronal excitability, energy metabolism, vascular regulation, and epigenetic control of gene expression, provide insight into the diversity of pediatric migraine clinical presentation.

3.1 Neuropeptides

Neuropeptides are endogenous peptide signaling molecules synthesized and released through regulated secretory pathways to influence neuronal activity and downstream biological responses. Despite the diversity of more than 100 identified neuropeptides, these signaling molecules share several conserved properties across the central nervous system and peripheral tissues (22).

Under normal physiological conditions, neuropeptides function as essential modulatory signaling molecules that regulate communication between the nervous, endocrine, and immune system. Through their ability to act alongside classical neurotransmitters, neuropeptides balance excitatory and inhibitory signaling while promoting longer-lasting responses in target cells. Beyond their roles within the central and peripheral nervous systems (CNS and PNS), neuropeptides are expressed in diverse tissues, including endocrine and immune cells, where they regulate nociception, neuronal regulation, autonomic and endocrine functions, and immune responses (22). Accordingly, the prevailing model of migraine pathophysiology proposes that neuropeptide-mediated dysregulation of the trigeminovascular system drives the initiation and maintenance of migraine (15).

In the diverse neuropeptide family, specific molecules have been identified as key mediators of migraine pathophysiology. The more extensively studied neuropeptides include calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38), vasoactive intestinal peptide (VIP), and substance P (SP) (15).

3.1.1 Migraine-Associated Neuropeptides

CGRP is widely regarded as a key mediator of migraine pathophysiology. Primarily synthesized and released from sensory nerve fibers, particularly C and A δ fibers, CGRP is considered the most potent microvascular vasodilator currently identified; it regulates local blood flow and mediates tissue responses (24). It is predominantly released from sensory afferent nerve terminals in blood vessels in the PNS, and it is expressed as primarily β -CGRP in the enteric nervous system, where it contributes to the regulation of gastrointestinal (GI) mobility and secretion. Within the CNS, CGRP and its receptors are distributed across multiple brain regions, where they are thought to modulate mechanisms implicated in migraine pathophysiology, including CSD, central sensitization, peripheral sensitization, and the processing of sensory symptoms (25).

PACAP-38 is a highly conserved neuropeptide belonging to the VIP/glucagon/secretin superfamily, widely distributed throughout the CNS and peripheral tissues, including the GI, endocrine, reproductive, and respiratory systems. Within the GI tract, the neuropeptide regulates the secretion of digestive hormones and enzymes, modulates smooth muscle contraction, controls local blood flow, and influences cell migration and proliferation (26). Within the CNS, PACAP fibers and their primary receptor, PAC1, are widely expressed in regions involved in autonomic regulation, circadian rhythms, stress responses, and pain processing; notably, PACAP signaling is localized within the hypothalamus, periaqueductal gray, locus coeruleus, trigeminal nucleus caudalis, and trigeminal ganglion, all of which have been implicated in migraine pathophysiology. This distribution suggests PACAP contributes to trigeminovascular signaling and nociceptive processing, providing a mechanistic basis for its role in migraine (27).

VIP is widely distributed throughout the CNS and PNS, as well as the GI tract. In addition to its roles as a neurotransmitter, VIP regulates vascular tone, smooth muscle contraction, hormone secretion, circadian rhythms, and immune responses through its inflammatory effects (28). Found in the trigeminal nerve, VIP is thought to contribute to migraine pathophysiology by promoting vasodilation, modulating neurotransmitter release, and regulating inflammatory signaling, thereby influencing trigeminovascular activation, pain sensitivity, and potentially migraine frequency and intensity (29).

SP is a neuropeptide expressed throughout the nervous and immune systems that regulate pain transmission, neurogenic inflammation, immune responses, and vascular function. Within sensory neurons, SP sensitizes postsynaptic neurons and facilitates the transmission of pain signals. During tissue injury, SP is released alongside CGRP, promoting neurogenic inflammation through mast cell degranulation, vasodilation, and recruitment of immune cells via cytokine-mediated signaling. These actions, known as the wheel & flare response, are implicated in the pathogenesis of several inflammatory disorders, including migraine (30).

3.1.2 Trends in Neuropeptide Levels

Alterations in neuropeptide signaling is a consistently observed biological feature associated with migraines. Although individual neuropeptides exhibit distinct physiological functions, dysregulation across these pathways converges on several interconnected mechanisms, including trigeminovascular activation, neurogenic inflammation, and central sensitization (14).

Among neuropeptide biomarkers, CGRP and PACAP-38 demonstrate the strongest association with pediatric migraine. Several studies have reported significantly higher plasma CGRP levels in children with migraine compared with controls without headache disorders, supporting its potential as an objective biomarker. CGRP's concentrations appear to fluctuate across different stages of migraine activity: Some studies have demonstrated elevated CGRP levels during the ictal phase (migraine attack phase) compared with healthy controls, and others have found no significant difference between ictal and interictal (between migraine attacks phase) CGRP levels and the controls (31, 32). Hence, elevations in CGRP levels may occur during periods of increased trigeminovascular activity and may depend on migraine phenotype and disease state, rather than remaining consistently elevated throughout the migraine cycle.

Changes in CGRP levels may also correspond with specific migraine phenotypes, particularly migraine with aura. In children with migraine with aura, ictal CGRP concentrations were reported as higher than interictal levels, and plasma CGRP levels during attacks were observed as greater than those in children with migraine without aura. These findings suggest CGRP may contribute to aura-related mechanisms, such as through interactions with CSD as Liu et al. proposes. During CSD, increased endogenous CGRP release has been observed, while CGRP receptor antagonists can reduce CSD-associated vascular and electrophysiological responses, supporting a role for CGRP signaling in the development of aura symptoms (31).

As Liu et al. notes, PACAP-38 similarly exhibits elevated plasma levels in pediatric migraineurs than in the healthy control during interictal and ictal periods, possibly due to delayed activation and sensitization of central trigeminovascular neurons (31). Hanci F et al. additionally noticed a negative correlation between interictal plasma PACAP-38 levels and attack frequency, further suggesting that PACAP-38 signaling may undergo dynamic regulation throughout the migraine cycle like CGRP (31, 32). Beyond its independent association with pediatric migraine (33), PACAP-38 has been shown to activate trigeminovascular neurons and stimulate CGRP release with the trigeminal nucleus caudalis, suggesting that PACAP-38 may function as an upstream modulator of CGRP-mediated signaling. Consistent with this relationship, Liu et al. found that combining plasma CGRP and PACAP-38 measurements provided greater diagnostic accuracy than either biomarker alone, demonstrating improved area under the curve (AUC) and specificity (31).

Found in the same family, VIP shares structural and functional similarities with PACAP-38, both able to activate VPAC1 and VPAC2 receptors and contribute to vascular and inflammatory signaling. Accordingly, elevated VIP levels have been observed in pediatric migraineurs, with studies reporting an increase in serum VIP concentrations during the ictal period compared with both the interictal periods and healthy controls. Additionally, elevated peripheral VIP levels have been reported in adults with chronic migraine, suggesting that persistent VIP dysregulation may be associated with migraine progression (34). Mechanistically, VIP may contribute to migraine

through its effects on vascular regulation and neurogenic inflammation: Prolonged VIP-mediated vasodilation has been associated with greater headache provocation, suggesting that sustained vascular activation may contribute to migraine pain (32). Therefore, VIP may function as a complementary neuropeptide pathway that influences migraine expression.

The diagnostic utility of SP remains less established. Compared with CGRP, PACAP-38, and VIP, fewer studies have found SP as a consistent biomarker in pediatric migraine initiation or progression. According to Hanci F et al., although one study identified elevated SP levels and suggested potential diagnostic value for pediatric migraine (35), other investigations have not consistently demonstrated significant differences in SP concentrations between ictal and interictal periods (34). There is little research on Substance P as a diagnostic biomarker in pediatric migraine, and Alpay et al. notes methodologic shortcomings in studies that pursue this topic (36). These findings suggest that while SP may contribute to migraine-related biological processes, particularly through pain transmission and neurogenic inflammation, its role as an objective biomarker requires further validation.

3.2 Inflammatory Biomarkers

Inflammation is the body's protective immune response activated by harmful stimuli, including tissue injury, infection, and cellular stress. Under normal physiological conditions, inflammation functions as a tightly regulated process that restores tissue homeostasis by initiating immune signaling, increasing local blood flow, recruiting immune cells, removing damaged cell components, and promoting tissue repair (37). However, when inflammatory responses become prolonged or improperly regulated, these same protective mechanisms can contribute to pathological processes, such as continued immune activation, vascular dysfunction, and increased sensitivity of surrounding tissues (37). Within the nervous system, this interaction between immune and neuronal pathways is referred to as neuroinflammation (38).

Inflammatory signaling is closely integrated with the activation of the trigeminovascular system (TVS), which leads to the release of neuropeptides and inflammatory mediators from peripheral nerve terminals. These mediators promote vasodilation, increased vascular permeability, and activation of nearby immune cells, including meningeal mast cells and dendritic cells. Activated immune cells subsequently release additional inflammatory cytokines and signaling molecules, creating a feedback loop that enhances trigeminal neuronal excitability. This interaction between neuronal activation, vascular changes, and immune signaling contributes to neurogenic inflammation, peripheral sensitization, and eventually central sensitization, all of which are key processes in migraine initiation and progression (38).

Among the inflammatory markers investigated in pediatric migraine, several molecules have emerged as potential indicators of altered immune activity. The following section examines interleukin-1 β (IL-1 β), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), and Pentraxin-3 (PTX3) as inflammatory biomarkers in pediatric migraine (38).

3.2.1 Migraine-Associated Inflammatory Biomarkers

IL-1 β is a pro-inflammatory cytokine that plays a central role in initiating and amplifying immune responses, primarily produced by activated immune cells, particularly monocytes and macrophages, in response to inflammatory stimuli (39). In the context of migraine, IL-1 β serves

as an inflammatory mediator and neurotransmitter that influences the onset and severity of migraines, both with and without aura (38).

IL-6 is a pleiotropic pro-inflammatory cytokine that regulates communication between the immune system, vascular system, and nervous system during inflammatory responses. It is produced by a wide variety of cells, including lymphocytes, macrophages, fibroblasts, and vascular endothelial cells, in response to inflammatory stimuli such as IL-1 β and TNF- α (40). In migraine pathophysiology, increased IL-6 activity may promote neuroinflammatory signaling, vascular dysfunction, and neuronal sensitization, presenting it as a candidate diagnostic biomarker (41, 42).

TNF- α is a pleiotropic pro-inflammatory cytokine that regulates acute and chronic inflammatory responses, released by innate immune cells, endothelial cells, neurons, and adaptive immune cells in response to inflammatory stimuli. TNF- α regulates downstream signaling pathways involved in immune activation, cell survival, and inflammatory mediator production. Within the nervous system, elevated TNF- α activity may contribute to increase neuronal excitability and pain sensitization through activation of microglia, regulation of glutamate signaling, and amplification of inflammatory cytokine networks, all of which are relevant in migraine pathophysiology (42).

CRP is an acute-phase inflammatory protein primarily synthesized by the liver in response to inflammatory signaling, particularly stimulation by IL-6. Under normal physiological conditions, CRP contributes to innate immune defense by recognizing damaged cellular components and pathogens, promoting their clearance through complement activation and interactions with immune cells. Because CRP concentrations rapidly increase in response to inflammatory stimuli and decrease following resolution, it is widely used as a marker of systemic inflammatory activity (44). In the context of migraine, CRP more likely reflects downstream systemic inflammation rather than a direct mediator of trigeminovascular activation.

PTX3 is a long pentraxin acute-phase inflammatory protein that belongs to the same protein family as CRP, but differs in its production and biological functions. PTX3 is produced locally by endothelial cells, macrophages, dendritic cells, neutrophils, and other immune cells following stimulation by inflammatory mediators such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). Beyond its role in immune activation, PTX3 is involved in vascular inflammation and endothelial regulation, processes that are recognized as relevant to migraine's neurovascular pathophysiology (45). Because PTX3 reflects local inflammatory activity within vascular and immune tissues, alterations in PTX3 levels may provide insight into neurovascular inflammation, endothelial dysfunction, and the inflammatory mechanisms associated with migraine susceptibility and progression (46).

3.2.2 Trends in Inflammatory Biomarker Levels

Evidence from pediatric populations suggests that pro-inflammatory cytokines may contribute to migraine pathophysiology, although individual cytokine patterns appear to vary across migraine phenotypes. In a study comparing children with migraine with and without aura to children with episodic tension-type headache, soluble tumor necrosis factor receptor 1 (sTNFR1), a marker of TNF- α pathway activation, was significantly elevated in children with migraine. Additionally,

TNF- α levels demonstrated a trend toward increased concentrations in migraine patients compared with controls. These findings suggest that TNF- α -related inflammatory signaling may contribute to pediatric migraine pathogenesis (47).

Beyond TNF- α -related signaling, studies investing other inflammatory biomarkers have demonstrated additional evidence of immune dysregulation in pediatric migraine: A recent study by Khan et al. reported elevated serum levels of CRP, IL-1 β , IL-6, and TNF- α compared with healthy controls, suggesting that inflammatory activation may represent a shared biological feature across migraine populations. IL-1 β and IL-6 may contribute to migraine-related pain through their ability to activate and sensitize meningeal and muscle nociceptors, increasing neuronal responsiveness within pain-processing pathways (48).

In pediatric migraine specifically, studies have reported significantly higher PTX3 levels in children with migraine, with elevated concentrations correlating with migraine severity. Because PTX3 is involved in acute and chronic inflammation as well as vascular dysfunction, increased PTX3 levels may reflect ongoing inflammatory activity and endothelial changes associated with pediatric migraine pathogenesis. Similarly, elevated CRP levels observed in migraine patients suggest that systemic inflammatory alterations may accompany migraine, although CRP likely reflects broader inflammatory activity rather than a migraine-specific process (48).

In contrast, CRP appears to have a more limited role as a migraine-specific biomarker. Although elevated CRP levels have been observed in migraine patients, its function as a broad marker of systemic inflammation may reduce its ability to reflect migraine-specific biological changes. Therefore, CRP may provide information regarding overall inflammatory status and may not accurately predict migraine severity or progression independently (46).

However, inflammatory biomarker patterns remain variable among patients. Differences in cytokine levels may reflect variation in migraine phenotype, attack frequency, and underlying biological pathways. Therefore, inflammatory biomarkers may be most valuable as components of broader biomarker profiles rather than isolated predictors. In pediatric migraine, further longitudinal studies are needed to determine whether inflammatory patterns can predict chronification, treatment response, or differences in clinical presentation.

3.3 Metabolic Biomarkers

Metabolic biomarkers represent measurable changes in small molecule metabolites that provide insight into the biological processes underlying health and disease. Unlike traditional biomarkers that reflect individual molecular pathways, because metabolites are downstream products of biological pathways, changes in their concentrations can indicate altered physiological states and disruptions in disease-related mechanisms. Endogenous metabolites are produced by the body or associated microbial communities and include molecules such as amino acids, carbohydrates, lipids, fatty acids, nucleotides, and organic acids (49).

Differences in metabolite profiles may indicate disruptions in cellular function, energy metabolism, or signaling pathways that contribute to disease susceptibility (49). In migraine, metabolic biomarkers are particularly relevant because altered metabolic processes may

influence neuronal excitability, cellular energy availability, and the ability of the nervous system to respond to physiological stress (50, 51).

The following section examines metabolic alterations associated with pediatric migraine, focusing on changes in tryptophan (Trp) metabolism, the kynurenine pathway, and gut microbiota-related metabolic processes.

3.3.1 Migraine-Associated Metabolic Biomarkers

The interaction between the CNS, enteric nervous system (ENS), GI tract, and intestinal microbiota forms the gut-brain axis, a complex communication network through which metabolic, neural, endocrine, and immune signals are exchanged. Microbiota-derived signals may directly affect the gut-brain axis through activation of vagal nerve pathways, while the CNS can also regulate microbial composition through autonomic nervous system (ANS) signaling and neuroendocrine pathways (52). Therefore, disruption of normal microbial composition, known as dysbiosis, may alter communication between the gut and brain and contribute to neurological conditions, including migraine.

In migraine, dysbiosis can influence the production of microbial-derived metabolites and inflammatory mediators, potentially increasing intestinal permeability and promoting systemic inflammation (53, 54). These changes may enhance trigeminovascular activation, neuroinflammation, and neuronal sensitization (53).

The gut microbiota may also influence migraine through regulation of metabolic pathways, particularly tryptophan metabolism. Tryptophan is an essential amino acid that is primarily metabolized through the kynurenine pathway under normal physiological conditions. Alterations in the kynurenine pathway have been identified in both migraine and functional GI disorders, suggesting that abnormal Trp metabolism may represent a shared mechanism linking gut dysfunction, inflammation, and neurological symptoms. Within migraine pathophysiology, kynurenine pathway metabolites may contribute to activation of pain pathways through modulation of trigeminovascular signaling and inflammatory responses (52).

3.3.2 Trends in Metabolite Levels

A study investigating gut microbiota and plasma Trp metabolism in pediatric migraines identified significant dysbiosis compared with healthy controls. Analysis of microbial composition revealed differences in both alpha and beta diversity, meaning that children with migraine had reduced microbial diversity and a different gut microbiota composition compared with healthy controls. Additionally, twenty-five bacterial genera demonstrate differential abundance between migraine and control groups, suggesting that changes in gut microbial composition may contribute to pediatric migraine pathophysiology (55).

Beyond overall microbial alterations, several bacterial genera involved in Trp metabolism showed significant differences between children with migraine and healthy controls. These findings support the hypothesis that gut microbiota may influence migraine development through regulation of Trp-derived metabolites, including Trp, serotonin (5-HT), kynurenine (KYN), kynurenic acid (KYNA), and quinolinic acid (QUIN). Because these metabolites regulate

neuronal activity, inflammatory signaling, and neurotransmitter pathways, disruption of their production may contribute to altered brain excitability and migraine susceptibility (55).

As per Liu et al.'s study, plasma analysis in children with migraine revealed altered Trp metabolism, characterized by decreased levels of neuroprotective metabolite KYNA and increased levels of 5-HT and QUIN (55). Reduced KYNA may contribute to neuronal hyperexcitability by decreasing inhibition of NMDA receptor activity and glutamate signaling, while elevated QUIN may promote neuroinflammation and pain sensitization through increased NMDA receptor activation. Additionally, decreased KYNA/KYN and increased QUIN/KYN ratios suggest dysregulation of the kynurenine pathway, with metabolism shifted away from protective KYNA production toward neuroactive QUIN production (55). Importantly, Liu et al. also demonstrated the diagnostic potential of these metabolic alterations, with the KYNA/QUIN ratio showing strong ability to distinguish pediatric migraine patients from healthy controls (AUC: 0.871); a combined model incorporated KYNA, QUIN, and 5-HT further improved diagnostic performance (AUC: 0.897), suggesting that tryptophan metabolisms may serve as promising metabolic biomarkers for pediatric migraines. Their metabolic changes may contribute to trigeminovascular activation, neuronal sensitization, and increased susceptibility to pediatric migraine.

3.4 Oxidative Stress Biomarkers

Oxidative stress refers to an imbalance between the production of reactive oxygen (ROS) and reactive nitrogen species (RNS) and the ability of the body's antioxidant systems to neutralize them. ROS and RNS are naturally produced during cellular processes and play central roles in normal cell signaling; however, excessive accumulation of these reactive molecules can overwhelm antioxidant defenses and lead to oxidative damage, altering cellular function and signaling pathways. Because mitochondria are a major source of ROS production during energy generation, mitochondrial dysfunction can increase oxidative stress and further impair cellular energy metabolism (56).

In high energy-demanding cells such as neurons, impaired mitochondrial function and increased oxidative stress may reduce adenosine triphosphate (ATP) production, disrupt ion regulation, and increase neuronal excitability (57). These changes may contribute to the abnormal neuronal activation and sensitization processes involved in migraine pathophysiology (58).

The following section explores growth differentiation factor 15 (GDF-15), fibroblast growth factor 21 (FGF-21), and hypoxia inducible factor 1 α (HIF-1 α).

3.4.1 Migraine-Associated Oxidative Stress Biomarkers

GDF-15 is a stress-responsive cytokine belonging to the transforming growth factor- β (TGF- β) superfamily, induced in response to cellular stress, inflammation, and tissue injury. Because mitochondrial dysfunction can activate cellular stress pathways, elevated circulating GDF-15 levels have been investigated as a biomarker of mitochondrial stress and impaired cellular function (59). In neurological disorders, elevated GDF-15 is particularly relevant because neurons rely heavily on mitochondrial energy production. Disruption of mitochondrial homeostasis may contribute to oxidative stress and increased neuronal excitability, processes implicated in migraine pathophysiology (60).

FGF-21 is a stress-inducible endocrine hormone primarily produced by the liver, with additional expression in skeletal muscle, adipose tissue, and the pancreas. FGF-21 regulates glucose and lipid metabolism and maintains energy homeostasis under normal conditions, but its expression increases in response to metabolic and mitochondrial stress (61). The relationship between FGF-21 and migraines is mostly indirect, driven primarily by metabolic processes (BMI) and the severity of headache pain rather than a unique, independent disease mechanism (60).

HIF-1 α is a transcription factor that regulates the cellular response to low oxygen availability (i.e. hypoxia). Under normal physiological conditions, HIF-1 α is rapidly degraded and maintained at low levels; however, hypoxia, mitochondrial dysfunction, oxidative stress, and changes in cellular metabolism stabilize HIF-1 α , allowing it to activate genes involved in energy metabolism and angiogenesis. Because the brain has a high demand for oxygen and energy, activation of HIF-1 α is thought to represent an adaptive response to metabolic stress. In migraine, increased HIF-1 α expression has been associated with neuronal sensitization (62).

3.4.2 Trends in Oxidative Stress Biomarker Levels

Recent evidence suggests that biomarkers of mitochondrial stress are consistently elevated in pediatric migraine. Compared with healthy controls, children with migraine demonstrated significantly higher serum concentrations of HIF-1 α , FGF-21, and GDF-15, with higher levels observed in patients with chronic migraine compared to episodic migraines and during ictal attacks rather than the interictal period. Among these biomarkers, Kilinc et al. concluded positive correlations between HIF-1 α and FGF-21 and migraine attack frequency, suggesting that increasing mitochondrial stress may be associated with greater disease burden. In addition, levels of HIF-1 α , FGF-21, and GDF-15 positively correlated with CGRP and PACAP-38, supporting the hypothesis that oxidative stress and mitochondrial dysfunction interact with trigeminovascular signaling in migraine pathophysiology. Kilinc et al. argues that, because children have higher energy demands due to their higher basal metabolic rate, they would require more oxygen consumption and hence excessive mitochondrial activity, potentially leading to the onset of migraine. Receiver operator curve (ROC) analysis further demonstrated that HIF-1 α , FGF-21, GDF-15, CGRP, and PACAP-38 each showed diagnostic potential for pediatric migraine, although these peptides had varying degrees of diagnostic value and larger longitudinal studies are needed to establish their clinical utility (63).

Adult data from a study conducted by Burrow et al. found that serum concentrations of GDF-15 and FGF-21 did not differ significantly between patients with episodic migraine and chronic migraine, suggesting that these biomarkers were not associated with migraine frequency or clinical subtype. Instead, GDF-15 and FGF-21 levels appeared to be more strongly influenced by factors such as age and body mass index (BMI), highlighting the need for further research to clarify their diagnostic and prognostic value. However, these findings should be interpreted with caution when applied to pediatric migraine, as the age-related differences in mitochondrial metabolism and disease pathophysiology may limit their generalizability (60).

3.5 Vitamin-Related Biomarkers

Vitamins involved in mitochondrial energy metabolism have also been investigated as potential metabolic biomarkers in migraine (58). Although they are not disease-specific biomarkers,

circulating vitamin concentrations may indicate underlying metabolic deficiencies that impair mitochondrial function and neuronal energy production. Because the brain has high energy demands, deficiencies in vitamins essential for oxidative phosphorylation and ATP synthesis may increase susceptibility to migraine by reducing cellular energy availability and increasing oxidative stress (18).

The following section observes the impact of coenzyme Q10 (CoQ10) deficiency on mitochondrial function, antioxidant defense, and migraine initiation and progression (64).

CoQ10, also known as ubiquinone, is a fat-soluble, vitamin-like molecule that functions as an electron carrier within the mitochondrial oxidative respiratory chain, facilitating ATP production and supporting cellular energy availability. Because neuronal function is highly dependent on mitochondrial energy production, reduced CoQ10 availability may contribute to impaired energy metabolism and increased oxidative stress, mechanisms that have been implicated in migraine pathophysiology. Hence, CoQ10 has been investigated as both a metabolic biomarker and a potential therapeutic target in migraine (65).

In a cohort of pediatric and adolescent migraine patients, a high proportion of individuals demonstrated reduced CoQ10 levels, with approximately 75% demonstrating low levels and about one-third showing levels below the established reference range; since CoQ10 serves as an antioxidant and plays a role in ATP production, decreased CoQ10 could indicate impaired mitochondrial energy production and reduced antioxidant capacity. Accordingly, Hershey et al. (2007) argues that migraine recurrence may increase a cycle where mitochondrial stress increases CoQ10 consumption and reduces the cell's ability to concentrate. However, similar to other mitochondrial biomarkers, CoQ10 deficiency is not specific to migraine, as Hershey et al. states, and likely represents a broader marker of metabolic stress rather than a disease-specific biomarker (66).

Future studies should determine whether reduced CoQ10 levels are specific to pediatric migraine or rather reflect metabolic or mitochondrial stress through a prospective, longitudinal study. Studies should also examine whether CoQ10 levels correlate with clinical characteristics, including migraine frequency, duration, severity, presence of aura, and treatment response. Incorporating additional measures of mitochondrial function and oxidative stress could further determine whether CoQ10 represents a broader mitochondrial phenotype. Such studies would help establish whether CoQ10 independently distinguishes pediatric migraine from other conditions and whether it has sufficient sensitivity and specificity to have diagnostic potential.

3.6 MicroRNA Biomarkers

MicroRNAs (miRNAs) are small, non-coding ribonucleic acid (RNA) molecules that regulate gene expression by inhibiting messenger RNA (mRNA) translation or promoting mRNA degradation. Through regulation of inflammation, metabolic, apoptosis, and neuronal function pathways, miRNAs have emerged as potential biomarkers in neurological disorders. A systematic review by Godzka et al. identified dysregulation of several miRNAs in migraine patients and highlighted their potential diagnostic value, with some miRNAs also influencing neuroinflammatory pathways and the expression of migraine-related peptides involved in

disease pathogenesis. However, current evidence remains limited, and further studies are needed to determine whether specific miRNA signatures can reliably serve as a diagnostic (20).

Evidence supporting the role of miRNAs in pediatric migraine has recently emerged. A pilot study evaluating children and adolescents with untreated migraine without aura demonstrated increased expression of hsa-miR-34a-5p and hsa-miR-375 in both serum and saliva compared with healthy controls and treated migraine patients. Hsa-miR-34a-5p has been linked to inflammatory responses and regulation of genes involved in GABAergic signaling, while hsa-miR-375 may influence neurotransmitter secretion and regulation of vesicular trafficking pathways. However, these findings are preliminary, as the study was limited by a small sample size and requires validation in larger cohorts. Further research is needed to determine whether specific miRNA signatures can reliably differentiate migraine from other pediatric headache disorders and whether they can predict disease severity or treatment response (67).

Most available miRNA studies have been conducted in adult populations as well, and the applicability of these findings to pediatric migraine remains unclear.

In adult migraine populations, additional evidence supports the potential role of miRNAs as biomarkers for disease identification, classification, and monitoring. Studies have demonstrated differential expression of multiple miRNAs between individuals with migraine and healthy controls, including altered levels of miR-155, miR-126, let-7g, miR-27b, miR-181a, miR-22, and miR-30a. Some miRNA alterations have also been associated with specific migraine characteristics, including aura status, chronic migraine development, and attack phases. For example, patients with chronic migraine have demonstrated increased expression of increased expression of miR-34a-5p and miR-382-5p similar to pediatric patients, which correlate with elevated levels of CGRP and PACAP-38. Additionally, differences in miRNA expression between ictal and interictal phases indicate that circulating miRNAs may reflect changes in migraine pathophysiology rather than simply disease presence (20).

3.7 Vascular Biomarkers

Vascular biomarkers have been investigated in migraine due to evidence of endothelial dysfunction and altered vascular regulation. Vascular involvement includes changes in dural vessels, where dilation and perivascular inflammation contribute to pulsatile pain, as well as alterations in cortical circulating during CSD in migraine with aura. The endothelium has also been implicated in migraine, with endothelial dysfunction proposed as a potential factor in migraine pathogenesis. However, it remains unclear whether endothelial dysfunction acts as a trigger for migraine attacks, contributes to migraine chronicization, or occurs as a consequence of repeated migraine episodes (68).

The relationship between vascular function and migraine pain is further supported by the role of the TVS, in which meningeal blood vessels are closely associated with nociceptive fibers originating from the trigeminal nerve and upper cervical spinal nerves. Changes in vascular dynamics, including mechanical vessel distension and the release of vasoactive mediators (e.g. nitric oxide and prostaglandins), can activate or sensitize meningeal nociceptors, promoting pain transmission through the trigeminovascular pathway. Activation of these pathways also

contribute to neurogenic inflammation through the release of vasoactive neuropeptides, further enhancing vasodilation (5).

Recent evidence in adult populations further supports the potential role of vascular and metabolic biomarkers in migraine pathophysiology. In patients with chronic migraine, elevated circulating levels of HIF-1 α and vascular endothelial growth factor-A (VEGF-A) have been identified, suggesting increased activation of hypoxia-responsive signaling and endothelial pathways. VEGF-A, a downstream target of HIF-1 α , contributes to endothelial activation, vascular permeability, and neurovascular remodeling, linking vascular dysfunction and migraine pathophysiology. However, these findings should be interpreted cautiously when considering pediatric migraine, as a majority of the evidence has been generated in adult chronic migraine populations; it remains unclear whether these biomarker alterations are present in children and adolescents.

4. Biomarker Heterogeneity and Clinical Presentation

Differences in neuropeptide signaling, inflammatory activity, mitochondrial function, and tryptophan metabolism may influence not only whether an attack occurs, but also the intensity and persistence of pain, sensory hypersensitivity, gastrointestinal symptoms, and central sensitization. Therefore, biomarker heterogeneity may provide a biological explanation for why children with the same migraine diagnosis can experience markedly different symptom profiles and levels of disability.

4.1 Headache Characteristics

4.1.1 Intensity and Pain

In children with migraine, CGRP concentrations were higher during the ictal period than during the interictal period, suggesting that CGRP activity may be related to the development of headache pain during attacks (31). In particular, when released from trigeminal sensory nerve endings, CGRP – and SP – can promote vasodilation and leakage of plasma proteins, producing neurogenic inflammation that contributes to headache pain. Activation of channels that detect pain and heat (e.g. TRPV1) can further stimulate the release of CGRP and SP, providing another mechanism through which trigeminal activation can enhance pain transmission.

Plasma levels of both PACAP-38 and VIP were found to be elevated during both ictal and interictal periods in pediatric migraineurs compared with healthy children, suggesting that alterations in these peptides may persist beyond individual migraine attacks. Interestingly, interictal plasma PACAP-38 levels were inversely associated with attack frequency, possibly due to alterations in cerebral energy metabolism (32). Together, these findings highlight alterations in pain-related neuropeptides that may reflect mechanisms underlying migraine intensity and the biological variability of pediatric migraine, suggesting its potential as biomarkers for understanding clinical presentation.

4.1.2 Episodic vs Chronic Migraines

Inflammatory and metabolic processes may also distinguish episodic migraine (EM) from chronic migraine (CM) and contribute to the increasing frequency of migraine attacks. In a study by Aczél et al., IL-6 and TNF- α were among the inflammatory markers most consistently

associated with higher-frequency or chronic migraine. Similarly, previous cohorts found that individuals who subsequently developed CM had higher baseline IL-6 and TNF- α concentrations than those who remained episodic. IL-6 can participate in inflammatory signaling and modulation of nociceptive pathways, and TNF- α can promote neurogenic inflammation and neuronal sensitization. Persistent activation of these pathways may therefore increase neuronal responsiveness and facilitate the maintenance of recurrent headache. However, Aczél et al. emphasized that directional longitudinal evidence demonstrating that these cytokines cause the transition from EM to CM remains limited (69).

Kilinic et al. demonstrated an association between mitochondrial stress-related signaling and migraine frequency. In a study of children with migraine, circulating levels of HIF-1 α , GDF-15, FGF-21, CGRP, and PACAP-38 were significantly higher in pediatric migraineurs than in healthy controls, and were higher in children with CM than those with EM; additionally, higher levels of these biomarkers were seen during migraine attacks than during attack-free periods in pediatric migraineurs. HIF-1 α and FGF-21 concentrations were also positively correlated with attack frequency, whereas the other measured biomarkers did not show the same association (63). Variations in inflammatory and metabolic biomarkers may thus be associated with differences in migraine frequency and chronicity, offering potential implications for biomarker-guided treatment approaches.

4.1.3 Presence of Aura

Migraine with aura (MA) is characterized by transient neurological symptoms that typically precede or accompany the headache phase, with visual, sensory, and language disturbances being among the most common manifestations. A central mechanism proposed to underlie aura is cortical spreading depression (CSD), a propagating wave of neuronal and glial depolarization followed by a period of suppressed cortical activity (70). CSD can produce transient deficits that may correspond to the neurological symptoms observed during aura and may subsequently activate trigeminal nociceptive pathways (71).

Gallai V et al. found higher ictal CGRP concentrations in those with MA than in those without aura, suggesting that increased CGRP signaling may be associated with the development of aura symptoms (31). This relationship may be influenced by age in pediatric patients as well – which proves particularly relevant when interpreting biomarker findings in pediatric migraine as neurovascular and trigeminovascular signaling systems undergo developmental changes during childhood and adolescence (72).

4.2 Associated Symptoms

4.2.1 Nausea and GI Issues

In children with migraine, plasma CGRP concentrations were positively correlated with nausea, consistent with the presence of CGRP within the enteric nervous system where it participates in the regulation of GI motility and secretion. In addition, stimulation of dural trigeminal afferents can similarly activate brainstem and hypothalamic regions involved in nausea and vomiting regulation. Neurogenic inflammation may further contribute to nausea through the release of SP and CGRP, which can promote mast cell degranulation and the subsequent release of histamine, serotonin, and pro-inflammatory cytokines such as TNF- α , IL-1, and IL-6. These mediators can interact with peripheral sensory and autonomic pathways and may alter GI

function, thereby contributing to nausea during migraine attacks (71). Interestingly, a study by Liu et al. suggested CGRP levels were not significantly associated with other GI manifestations, including vomiting and abdominal pain. Limited clinical information and potential unmeasured confounding factors may have influenced these observed relationships (31).

4.2.2 Phobias

Photophobia may involve several interconnected pathways, including trigeminal afferents, the trigeminal nucleus caudalis, posterior thalamus, hypothalamus, and visual and somatosensory cortices. The posterior thalamus, in particular, is thought to process light-related signals from retinal ganglion cells and nociceptive signals from the dura together, offering a possible explanation for why photophobia is a symptom of migraines. Additionally, CGRP receptors are present on thalamic neurons responsive to trigeminovascular input, suggesting the neuropeptide may contribute to photophobia by modulating pain and sensory-processing pathways. Interactions between CGRP-mediated pain pathways and limbic circuits may further contribute to the emotional discomfort and strong preference for darkness experienced during migraine (73).

4.3 Allodynia and Central Sensitization

Sustained release of neuropeptides, such as CGRP and PACAP, can increase excitability within central pain-processing regions, including the caudal trigeminal nucleus (TNC) and thalamus (71). CGRP lowers the activation threshold of channels like TRPV1, increasing the sensitivity of nociceptors to thermal and mechanical stimuli. Within the TVS, the release of inflammatory mediators during a migraine attack can enhance this sensitization, lowering mechanical activation thresholds and increasing spontaneous neural activity. CGRP may additionally contribute by modulating nociceptive signaling and amplifying the responsiveness of pain-sensory pathways (74). Nitric oxide may further promote the release of biomarkers like CGRP, SP, and glutamate through retrograde signaling, creating a positive feedback loop that contributes to both peripheral and central sensitization (71). In turn, central sensitization lowers sensory thresholds and amplifies responses to normally innocuous stimuli, contributing to sensory symptoms such as cutaneous allodynia, photophobia, and phonophobia (75).

Beyond neuropeptide-mediated mechanisms, alterations in metabolic pathways may also provide potential biomarkers of migraine-related neuronal sensitization: A recent study of pediatric migraine patients found significantly higher levels of kynurenine (KYN), kynurenic acid (KYNA), and the KYN/tryptophan (TRP) ratio compared with controls (76).

The findings presented in this section demonstrates that variations in neuropeptide, inflammatory, and metabolic biomarkers are associated with clinical features of pediatric migraine. The predominantly associative nature and the potential effects of age, attack phase, and other clinical factors limit conclusions regarding whether these biomarkers directly drive specific migraine phenotypes. Instead, they support the potential value of biomarker profiling for further characterizing the biological heterogeneity of pediatric migraine.

5. Biomarker-Informed Pharmacologic Treatment Strategies

Mechanistic variation in pediatric migraine onset and progression is evidence in the heterogeneous therapeutic responses observed among pediatric patients. Accordingly,

biomarker profiles may inform treatment selection beyond symptom-based approaches by identifying dysregulated pathways that may be particularly amenable to targeted pharmacologic intervention.

5.1 Neuropeptide-Targeted Therapies:

Among the biomarker-informed strategies currently investigated, neuropeptide-targeted therapies offer one of the strongest mechanistic rationales. Neuropeptides intervene relatively close to trigeminal processes that generate and manage migraine pain and appear to intersect with and correlate with broader biomarker patterns. Accordingly, CGRP and PACAP-38 have emerged as central focuses of current migraine treatment development, with CGRP-targeted therapies now an established component in migraine prevention and PACAP-targeted therapies as a promising treatment method (77).

The “gepant” class consists of CGRP receptor antagonists developed as an alternative to triptans for acute migraine treatment, with efficacy comparable to that of triptans. Their therapeutic effects arise from inhibition of CGRP signaling in the TVS rather than pure vasoconstriction, thereby reducing migraine-related nociceptive signaling, vasodilation, and neurogenic inflammation without unnecessarily constricting otherwise healthy blood vessels. Depending on the agent, gepants can be used for either abortive or prophylactic treatment. Acute-use agents are administered at migraine onset to interrupt CGRP signaling and alleviate symptoms, while preventive gepants (e.g. rimegepant, atogepant) are taken regularly to reduce the frequency of future attacks through sustained inhibition of the same pathway. Newer gepants were developed to avoid the hepatotoxic metabolite associated with earlier agents, such as telcagepant, overcoming a major limitation that hindered the early development of the drug class (78).

Given that a portion of CGRP receptors exists outside of the blood brain barrier (BBB), large molecules with limited BBB penetration represent a viable approach to migraine therapy. Of these larger molecules are monoclonal antibodies (mAbs) targeting CGRP itself or the CGRP receptor with high target specificity, minimizing the off-target effects associated with small-molecule therapies. Their long half-lives allow less frequent dosing as well (78). However, the use of anti-CGRP/receptor mAbs in pediatric and adolescent migraine remains cautioned due to limited clinical evidence, with the Pediatric and Adolescent Headache Special Interest Group of the American Headache Society recommending against unrestricted use until the results from ongoing randomized controlled trials in these age groups become available; For instance, their use in pediatric populations warrants caution given the physiological roles of CGRP in vasodilation and bone formation (77). In selected patients with refractory migraine, mAbs may nevertheless be considered after the failure of two preventive medications administered for 2-3 months at the lowest effective dose and shortest appropriate duration. Until pediatric trial data are available, anti-CGRP/receptor mAbs therefore remain a cautious, individualized option for patients younger than 18 years and are not yet reimbursed for pediatric migraine in several countries (79).

PACAP has emerged as a promising although considerably less established therapeutic target. Clinical trials of mAbs targeting either PACAP or its PAC1 receptor have produced differing results, with some studies demonstrating significant reductions in monthly migraine days while

others have failed to show a significant preventive benefit compared with placebo. Additionally, PAC1-targeting mAbs remain under investigation, including in patients with treatment-resistant migraine (29). Further research, including larger trials, identification of responsive patient subgroups, optimization of dosing, and evaluation of combination therapies, is needed to clarify their therapeutic potential (80). Importantly, PACAP-targeting therapies have not yet been evaluated in pediatric populations, with current clinical trials restricted to adults, leaving their safety and efficacy in children and adolescents unknown (81).

5.2 Metabolic and Mitochondrial Pathway-Targeted Therapies:

Mitochondrial and metabolic abnormalities in pediatric migraine provide potential therapeutic targets focused on reducing neuronal susceptibility and limiting downstream pathophysiological effects.

Nutraceuticals such as riboflavin (vitamin B2), vitamin D, coenzyme Q10 (CoQ10), and magnesium have been investigated as mitochondrial-supportive therapies. In a randomized controlled trial conducted by Slater et al., administration of CoQ10 had been shown to insignificantly decrease attack frequency and intensity with no adverse events, presenting it as a potential prophylactic therapy for migraine (82). Riboflavin may help prevent migraine attacks by supporting mitochondrial function, reducing neuroinflammation, and helping regulate blood vessels and neuronal function. However, due to conflicting results and limited evidence, its effectiveness in pediatric migraine prevention and the factors that predict treatment response remains unclear (48).

Vitamin D has also been investigated as a potential therapeutic agent due to its neuroprotective and immunomodulatory properties as a neurosteroid. Studies have demonstrated an association between vitamin D deficiency and primary headaches in children, and a study conducted by Fallah et al. found vitamin D supplementation alongside conventional preventive therapy may be more effective than conventional therapy alone in reducing headache frequency and improving daily living (83).

Magnesium may similarly influence several mechanisms involved in migraine pathogenesis, with its deficiency associated with altered neurotransmitter release, CSD, and increased platelet aggregation. However, evidence for its effectiveness in pediatric migraine as a supplement remains inconsistent, with randomized controlled studies showing no significant differences in primary outcomes, while other studies have reported reductions in pain intensity among children receiving magnesium as part of migraine management (48).

5.3 Diet and Lifestyle Management:

Growing evidence suggests that specific components of the gut microbiota may influence migraine through the gut-brain axis, particularly by modulating neuroinflammation and pain-related signaling. In a recent study by Fan et al., pediatric patients receiving *B. longum* experienced improvements in pain intensity, attack frequency, and headache days. *F. prausnitzii* has also been associated with reduced migraine severity and may contribute to an anti-inflammatory intestinal environment through increased production of beneficial metabolites like butyrate. Similar benefits were not observed with a combination of *B. bifidum* and *L.*

acidophilus; these results suggest the effects of probiotics on migraine may depend on the specific bacterial strain rather than probiotic supplementation as a whole. (84).

Although no single dietary pattern has been established as universally effective, maintaining a balanced diet and healthy weight may be associated with improved migraine outcomes. Irregular meals and skipping meals, as common in adolescents, have been linked to more frequent and severe migraine attacks and may trigger headaches accompanied by mild nausea. Identifying and avoiding individual dietary triggers (e.g. chocolate, caffeine, alcohol, nitrites, aspartame, and gluten) may also help prevent migraine episodes in susceptible children. More restrictive approaches, such as ketogenic diets, have demonstrated reductions in neuroinflammation and CSD in adults, although evidence in pediatric migraine remains limited. Adequate hydration with non-caffeinated fluids is likewise recommended as part of comprehensive migraine management, as maintaining appropriate fluid balance may support cerebral perfusion and electrolyte homeostasis (85).

Overall, emerging treatment strategies target several biological pathways implicated in pediatric migraine, including neuropeptide signaling, mitochondrial function, metabolism, and the gut-brain axis. Although these approaches demonstrate potential, limited, and inconsistent pediatric evidence prevents most biomarkers from being used as validated predictors of treatment response.

6. Potential Multimarker Diagnostic Platforms for Pediatric Migraine

Rather than relying on individual biomarkers, future approaches may benefit from multimarker panels that could potentially predict treatment response, identify children at greater risk of progression from EM to CM, and monitor treatment effectiveness over time – thus contributing to more individualized treatment.

The integration of multiple biomarkers into a minimally invasive diagnostic platform may offer a potential approach for improving the diagnosis and longitudinal monitoring of pediatric migraine. Given its established role in the trigeminovascular system, CGRP may serve as a potential target for such a diagnostic. In adult migraineurs, CGRP has been detected in tear fluid at concentrations about 140-fold higher than those measured in plasma in both healthy controls and migraineurs, presenting tear fluid as a promising, minimally invasive compartment for monitoring CGRP activity (86). According to Kamm et al., CGRP levels fluctuate according to the attack phase – with higher levels during the ictal phase compared to interictal – and the presence of medication (namely triptans and/or NSAIDs) – with medicated ictal CGRP levels similar to baseline levels.

Accordingly, tear-fluid CGRP measurements could be adapted into a portable, point-of-care or at-home system that enables repeat assessment outside of a laboratory setting, where migraine prediction hinges on the concentration of CGRP measured. Novikov et al. described such an approach through a portable, semiquantitative device that combined tear collection with an immunochromatographic test strip containing CGRP-specific antibodies, exemplifying the feasibility of translating laboratory-based CGRP measurements into a portable. Although this technology has not been validated as a pediatric migraine diagnostic, its adaptation based on pediatric physiology could provide a minimally invasive means of monitoring CGRP dynamics in

children and adolescents, allowing tracking of biomarker levels (87). The mechanism for this model is explored below in Figure 3.

Figure 3. Proposed mechanism of a portable, tear-fluid CGRP detection system for migraine monitoring. The model illustrates tear collection via a Schiermer test strip and follows immunochromatographic detection of CGRP using CGRP-specific antibodies, providing a semiquantitative measurement of CGRP concentration that could potentially be used for repeated, minimally invasive monitoring of migraine-related biomarker dynamics.

Other migraine-associated biomarkers could potentially be assessed through dermal interstitial fluid (ISF). Microneedle-based diagnostics centers around the use of skin interstitial fluid (ISF) as an accessible source of biomarkers for disease or drug detection and monitoring. Originating from capillary filtration into the intracellular dermal space, ISF serves as an alternative biofluid to blood serum and plasma. It is able to be accessed with minimal damage to blood vessels and carries a reduced risk of infection (88). Existing microneedle (MN) platforms have demonstrated the detection of metabolic biomarkers and proteins in ISF, with recent advances integrating the technology with electrochemical sensors and wearable electronics for continuous monitoring (89). This approach could therefore be adapted to monitor the migraine-associated metabolic, mitochondrial, oxidative-stress, and select inflammatory biomarkers (e.g IL-6) identified in the preceding sections. A hollow or porous MN patch could periodically sample dermal ISF and either transfer the sample to a portable assay or directly quantify selected biomarkers through an integrated sensor, allowing repeated measurements of multiple biomarkers over time (90). The mechanism for this model is explored further in Figure 4. Although the use of MN-based ISF monitoring has not been established for pediatric migraine, its minimally invasive nature and demonstrated capacity for continuous biomarker monitoring present it as a potential application.

Figure 4. Proposed mechanism of microneedle-based dermal interstitial fluid (ISF) monitoring for pediatric migraine-associated biomarkers, namely metabolic, inflammatory, and oxidative-stress/mitochondrial related biomarkers. The model illustrates the minimally invasive collection of ISF via a hollow or porous microneedle patch, followed by direct detection using an integrated electrochemical sensor. This approach could potentially enable repeated or continuous monitoring of the aforementioned biomarkers to characterize changes in biomarker levels associated with migraine and predict the onset of pediatric migraine attacks.

7. Conclusion

Pediatric migraine is currently diagnosed primarily through clinical symptoms, an approach that does not fully capture the biological heterogeneity and symptomatic variation underlying different migraine phenotypes. This review examined how variations across biomarker classes may contribute to differences in the physiology and mechanism underlying pediatric migraine and whether these biomarkers may inform treatment. Across the literature, neuropeptides, inflammatory mediators, metabolic and mitochondrial proteins, oxidative-stress markers, vitamin-related biomarkers, microRNAs, and vascular biomarkers have each demonstrated associations with aspects of pediatric migraine pathophysiology. However, findings remain heterogeneous, and no individual biomarker has demonstrated sufficient reliability or specificity to replace current clinical diagnostic approaches.

Biomarker profiles may have the potential to improve patient stratification, identify children at greater risk of migraine chronification, predict treatment response, and support more individualized treatment selection. A potential approach is integrating multiplex biomarker-sensing into microneedle patches to offer real-time detection of inflammatory, oxidative stress, and metabolic biomarker levels, offering real-time, longitudinal tracking of broader physiological shifts (e.g. systemic inflammation). In combination, a point-of-care tear test as an immunochromatographic test strip for tear CGRP quantification further allows confirmation of trigeminovascular activation through localized CGRP strikes. This dual-platform allows healthcare providers and pediatric patients to predict impending attacks during the prodromal phase, identify children at heightened risk for disease progression, and allow more targeted therapeutic interventions. Prototypes and clinical trials testing targeted therapies and diagnostics for pediatric migraineurs remain limited, despite growing interest in the field. Most proposed biomarkers have not yet undergone sufficient validation to support routine clinical decision-making, and differences in study populations, methodologies, and biomarker measurement may contribute to inconsistent findings. Future research hence should prioritize larger, longitudinal, pediatric-specific studies to determine how biomarker levels change across migraine states and over the course of disease progression. Standardization of sampling and measurement techniques will improve comparability across studies, and validation of multimarker panels may be more informative in diagnosis.

Biomarkers are unlikely to replace existing symptom-based diagnostic criteria dictated by the ICHD-3. Instead, biomarker profiling may complement clinical assessment by identifying biologically distinct migraine subtypes and providing additional information to guide treatment selection.

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Abbreviations

5-HT: serotonin, 5-hydroxytryptamine
ATP: Adenosine triphosphate
AUC: Area under the curve
BMI: Body mass index
CGRP: Calcitonin gene-related peptide
CM: Chronic migraine
CNS: Central nervous system
CoQ10: Coenzyme Q10
CRP: C-reactive protein
CSD: Cortical spreading depression
EM: Episodic migraine
ENS: Enteric nervous system
ET-1: Endothelin-1
FGF-21: Fibroblast growth factor-21
GABA: Gamma-aminobutyric acid
GDF-15: Growth differentiation factor-15
GI: Gastrointestinal
HIF-1 α : Hypoxia-inducible factor-1 alpha
IL: Interleukin
ISF: Interstitial fluid
KYN: Kynurenine
KYNA: Kynurenic acid
MA: Migraine with aura
miRNA: MicroRNA
MN: Microneedle
mRNA: Messenger RNA
PACAP-38: Pituitary adenylate cyclase-activating polypeptide-38
PNS: Peripheral nervous system
PTX-3: Pentraxin-3
QUIN: Quinolinic acid
ROC: Receiver operator curve
ROS: Reactive oxygen species
RNS: Reactive nitrogen species
TGF- β : Transforming growth factor- β
TNC: Caudal trigeminal nucleus
TNF- α : Tumor necrosis factor- α
Tryptophan: Trp
TVS: Trigeminovascular system
VEGF: Vascular endothelial growth factor (VEGF)
VIP: Vasoactive intestinal peptide
SP: Substance P
sTNFR1: Soluble tumor necrosis factor receptor 1
NPY: Neuropeptide Y

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