

Piezo channels in migraine and trigeminal pain syndromes: a systematic review of their role in pain pathways

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ABSTRACT

Piezo channels, a class of mechanosensitive ion channels, have emerged as key players in sensory transduction. Piezo1 and Piezo2 have been implicated in various physiological processes, including touch sensation and nociception. Their association with migraine and their role in pain involving trigeminal nerve have gained significant research attention. Following PRISMA guidelines, we performed a systematic review of the literature, on the role of Piezo1 and Piezo2 channels in migraine and trigeminal pain. From PubMed, Cochrane Library, and Scopus, we deemed 20 studies published between 2014 and 2023 appropriate to be included in this review. Piezo1 emerges as a key player in migraine pathogenesis, contributing to meningeal nociception and pain generation. In trigeminal pain syndromes, Piezo channels, particularly Piezo2, have a role in various pain conditions, from corneal nociception to dental and orofacial pain. Mechanistic insights provide potential therapeutic targets for migraine and other pain conditions involving trigeminal nerve. This systematic review accentuates the emerging significance of Piezo channels in migraine and trigeminal-associated pain, underscoring cross-associations that interconnect Piezo channels, migraine, and trigeminal neurons, as well as suggesting promising avenues for targeted therapeutic interventions and future research directions.

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Introduction

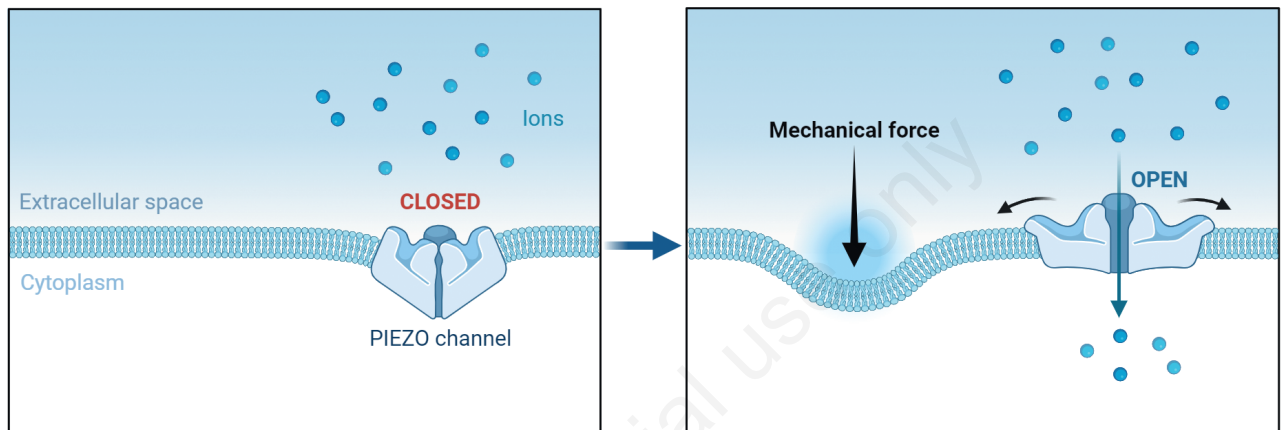
The pathogenesis of migraine and other pain syndromes involving trigeminal transmission remains enigmatic concerning the diagnostic and therapeutic challenges they pose along with their complexed pain transmission pathways.¹ Recent data in the literature has hinted at a potential explanation that could unify migraine with other trigeminal-mediated pain syndromes, pointing to the role of Piezo channels, a class of mechanosensitive ion channels which have emerged as key players in sensory transduction mechanisms. Within this family of transmembrane proteins, Piezo1 and Piezo2 are two distinct subtypes which have been studied thoroughly.²⁻⁴ Their involvement in various physiological processes, including touch sensation, proprioception, and nociception, has garnered substantial research interest.^{5,6}

The discovery of the Piezo ion channel gene family took place in 2010 by Professor Ardem Patapoutian and led to the acquisition of the Nobel Prize in Physiology or Medicine 2021 jointly with David Julius. These ion channels convert mechanical forces into biological signals and because of this they are considered today as key factors of mechano-transduction and pain signaling (Figure 1). As is frequently the case in science, discoveries have a profound influence on fields of study other than their own. While the discovery of Piezo channels has revolutionized our knowledge of touch and proprioception, it has also produced unexpected insights into mechanosensation that is not limited to the skin, such as immune system regulation and plant root functions.⁷ Piezo1 and Piezo2 were found to be widely expressed in a variety of tissues and organs such as the nervous system, stomach, lungs, bladder, intestines, and blood vessels.⁷ Therefore, the true significance and future of Piezo channels finding may lay well beyond the questions it was originally intended to be answered.

Concerning their biochemical constitution, Piezo channels are characterized by their distinctive structural layouts and efficient mechanical systems.⁸ Both Piezo1 and Piezo2 have a distinct 38-

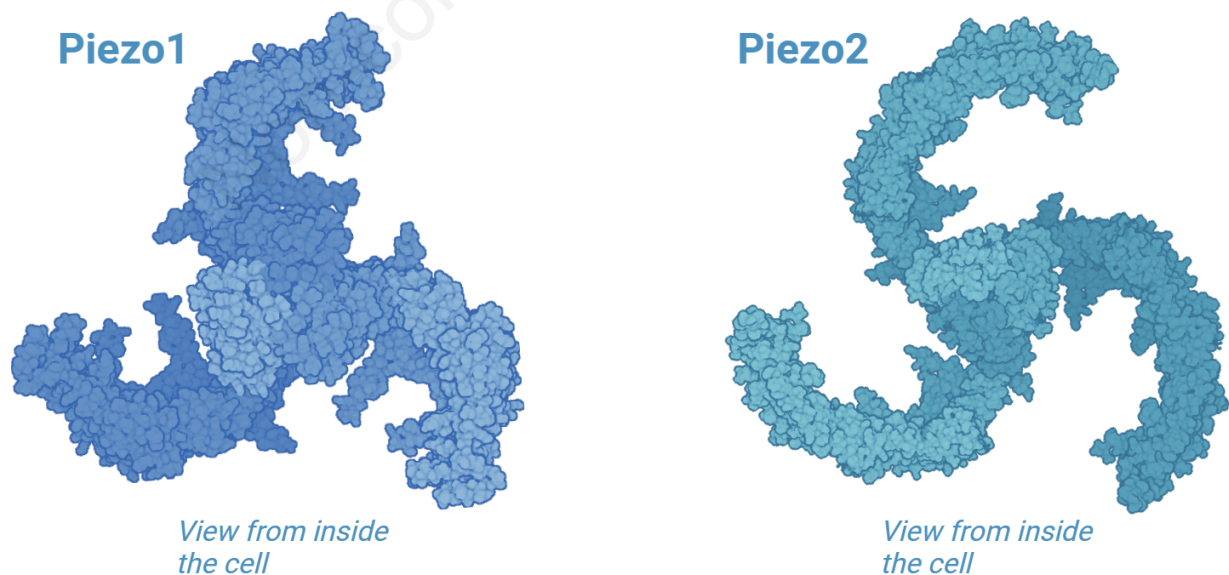
transmembrane (TM) helix topology and constitute of a homotrimeric propeller-like structure with three peripheral mechanosensing blades and a central ion-conducting pore.⁸ The unusually curved TM regions of the three blades shaped structure, form a signature nano-bowl design of a total of 114 TM helices that is able to produce a significant in-plane membrane area expansion and provide Piezo channels with exceptional mechanosensitivity (Figure 2).⁸ More precisely, Piezo1 channel is important in the initiation of Ca^{2+} signaling in a variety of non-excitable cell types and Piezo2 is primarily involved in the senses of touch, pain, balance, breath, blood pressure, and bladder fullness in sensory neurons and specialized cell types such as.⁸

Piezo channels are now being investigated due to their potential association with migraine and their role in trigeminal neurons, where pain pathways demonstrate complex interrelations. Migraine, a prevalent neurological disorder marked by recurrent headache episodes and associated symptoms, imposes substantial disruptions on individuals' quality of life.⁹ The most current research on Piezo channels as the vascular and neural sensors of intracranial mechanical pressures, offers a new perspective on the molecular mechanisms behind meningeal nociception and migraine pain.¹⁰ Trigemino-vascular nociceptive system in meninges is considered the origin site of migraine and its components, such as aura.¹⁰ Cortical spreading depres-



Piezo1 and Piezo2 are both mechanically-activated ion channels; these ion channels convert mechanical forces into biological signals. Piezo channels respond to mechanical stimuli by changing their structure and allowing the flow of extracellular ions into the cytoplasm.

Figure 1. Piezo channels: how do they allow mechanosensation? (created with BioRender.com).



Both Piezo1 and Piezo2 have a distinct 38-transmembrane (TM) helix topology and constitute of a homotrimeric propeller-like structure with three peripheral mechanosensing blades and a central ion-conducting pore.

Figure 2. Molecular structures of piezo channels (created with BioRender.com).



sion, a wave of strong depolarization of cortical neurons and glial cells, leads to meningeal neurogenic inflammation and dilation of the dural vessels that could further sensitize Piezo channels.¹⁰ Severe migraine symptoms, such as mechanical hyperalgesia, may be the result of these mechanosensitive systems.¹⁰ Besides, it has been shown that meningeal afferents' expression of the Piezo1 receptor enables these neurons to react to mechanical force.¹¹

It is also known that primary sensory neurons, whose cell bodies are located in the trigeminal ganglia and dorsal root ganglia (DRG), are the key mediators of mechanosensation in mammals.¹² An extensive transcriptome analysis on RNA-sequencing data from the human trigeminal ganglia has identified Piezo1 and Piezo2 channels.¹³

It is essential to examine more thoroughly and systematically the results obtained from research to this date, to argue whether Piezo channels have indeed an important role in the pathogenesis of migraine and other painful syndromes transmitted *via* the trigeminal nerve.

This systematic review aims to comprehensively explore the existing literature on the involvement of Piezo1 and Piezo2 channels in migraine and their role in trigeminal pain syndromes. By systematically analyzing and synthesizing relevant studies our goal was to unveil potential cross-associations, interactions, and mechanistic insights that interconnect Piezo channels, migraine, and trigeminal pain syndromes. Furthermore, we assess possible therapeutic implications of these findings and chart future research directions in this evolving field.

Methods

Protocol registration

This review was registered in PROSPERO, an international prospective register of systematic reviews, under registration number CRD42023481700.

Literature search strategy

A systematic literature search, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,¹⁴ was performed in the following databases: PubMed, Cochrane Library, and Scopus in July 2023. In the Cochrane Library we did not identify any previous Systematic Reviews concerning our research topic. The medical subject heading (MeSH) terms used for PubMed were (“Piezo” OR “Piezo channels” OR “Piezo1” OR “Piezo2”) AND (“Pain” OR “Migraine” OR “Trigeminal”). For our search in Scopus, we performed logic combinations of the above MeSH terms (e.g. “Piezo AND Pain”, “Piezo1 AND Migraine”, “Piezo2 AND Trigeminal”). The reference lists of the articles that met the eligibility criteria were further screened to identify additional eligible studies.

Eligibility criteria

Only original studies published in English were selected. The aim of this review was to identify studies concerning the association between Piezo1 and Piezo2 channels and specific types of pain. All articles exploring migraine pain or the trigeminal neurons and studied their relationship with Piezo1 and Piezo2 channels were included. Articles that did not include Piezo channels, did not refer to migraine or trigeminal neurons or were not original studies in English were excluded from this review.

All article titles and abstracts were reviewed by two authors (TA, MK) after the removal of duplicates. A third author (MR) was brought in to address any differences of opinion. The two authors (TA and MK) reevaluated all the articles that seemed to be eligible as full texts, and a third author (MR) resolved any conflicts.

Quality assessment of included studies

We utilized the SYRCLE Risk of Bias (RoB) tool for animal studies¹⁵ to perform quality assessment of the animal studies that were included in our review.¹⁶⁻³⁴ This tool, adapted from the Cochrane RoB tool, assessed RoB for 10 types of bias/ domains: i) selection bias/sequence generation; ii) selection bias/baseline characteristics; iii) selection bias/allocation concealment; iv) performance bias/random housing; v) performance bias/blinding; vi) detection bias/random outcome assessment; vii) detection bias/blinding; viii) attrition bias/incomplete outcome data; ix) reporting bias/selective outcome reporting; and x) other sources of bias. A table of this assessment is presented in (Figure 3).

	Random sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Mikhailov (2019)	?	?	?	?	?	?	?	?	?	?
Dulgorukova (2021)	?	+	?	+	?	?	?	+	?	?
Krivoshein (Dec. 2022)	?	+	?	?	?	?	?	?	?	?
Mikhailov (2022)	?	?	?	?	?	?	?	?	?	?
Della Pietra (2023)	?	+	?	?	?	?	?	?	?	?
Bron (2014)	?	?	?	-	?	?	?	?	?	?
Alamri (2015)	?	?	?	?	?	?	?	?	?	?
Fernández-Trillo (2020)	?	?	?	?	?	?	?	?	?	?
Emrick (2020)	?	?	?	?	?	?	?	?	?	?
Lee (2019)	?	?	?	?	?	?	?	?	?	?
Roh (2020)	?	?	?	?	?	?	?	+	+	?
Sato (2018)	?	?	?	?	?	?	?	?	?	?
Cho (2022)	?	?	?	?	?	?	?	?	?	?
Liu (2021)	?	?	?	?	?	?	?	?	?	?
Luo (2021)	?	?	?	?	+	?	+	?	?	+
Bai (2020)	?	?	?	?	?	?	?	+	?	+
Chung (2016)	?	?	?	?	?	?	?	?	?	?
Krivoshein (Oct. 2022)	?	?	?	?	?	?	?	?	?	+
Schneider (2019)	?	?	?	?	?	?	?	?	?	?

Figure 3. SYRCLEs risk of bias assessment tool. ?, unclear risk of bias; +, low risk of bias; -, high risk of bias.

Process of data extraction

Following the identification of the articles that met our eligibility criteria, the relevant data was extracted from each paper. Two reviewers (TA, MK) extracted data independently in a standardized data extraction Excel sheet including: name of the first author, study characteristics, year of publication, type of receptors that were studied and an association with migraine or trigeminal neurons/ganglia. Any disagreement or queries were resolved by discussion between authors, followed by consulting an external reviewer (MR), if necessary.

Results

Our search identified 453 articles. After duplicate detection and removal, 412 of them underwent title and abstract screening; 296 of them were removed because they did not adhere to the objectives of this review. Ninety-eight articles were excluded after reading the full text. By searching the reference lists of the retrieved papers, 2 extra articles were identified. Ultimately, a total of 20 articles, published between 2014 and 2023, were included in the review. The selection process is shown in (Figure 4). Table 1 is summarizing the results of each study.

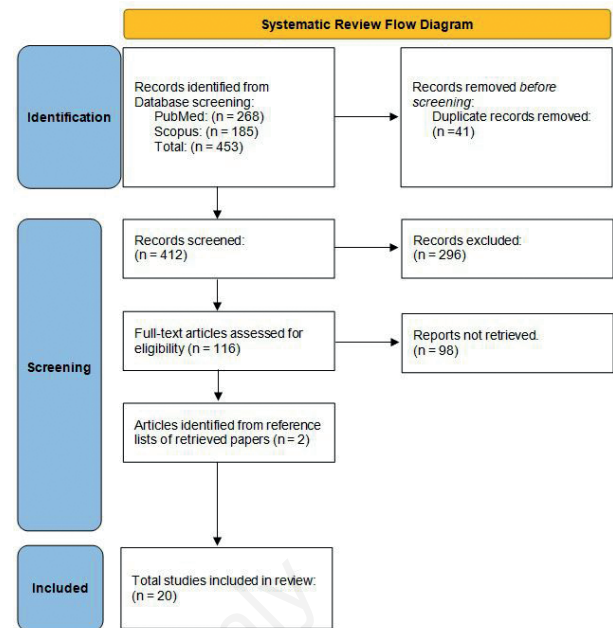


Figure 4. PRISMA (Preferred Reporting Items for Systematic Reviews) flow diagram.

Table 1. Summary table of the role of Piezo1, Piezo2, and both Piezo1 and 2 in migraine and in trigeminal neurons as found in the articles reviewed.

	Role of Piezo1 and Piezo2	Role of Piezo1	Role of Piezo2
In migraine	Activation of Piezo receptors stimulates the release of the migraine mediator calcitonin gene-related peptide (CGRP) in trigeminal neurons and peripheral meningeal nerve fibers. This suggests that Piezo1 and Piezo2 channels contribute to pain generation in the meninges by increasing nearby neurons' sensitivity to CGRP.		Mikhailov <i>et al.</i> (2019)
In migraine		Mechanotransduction in migraine pathogenesis and possible control of vascular tone in a migraine attack through dilatation of the arteries innervated by trigeminal afferents.	Dolgorukova <i>et al.</i> (2021)
In migraine		Lack of a sex difference in the Piezo1 channel activation in meningeal afferents.	Krivoshein <i>et al.</i> (2022)
In migraine		Piezo1 functionality in trigeminal neurons rapidly enhances during a migraine attack. Trigeminal neurons exhibited few functional Piezo1 receptors but a lot of Piezo-coding mRNA suggesting reverse capacity.	Mikhailov <i>et al.</i> (2022)

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Table 1. Continued from previous page.

	Role of Piezo1 and Piezo2	Role of Piezo1	Role of Piezo2
In migraine		Activation of Piezo1 channels in trigeminal neurons and SGCs produces extended labeling with FM1-43 dye. This persistent labeling can be utilized to track both current and past Piezo1 channel activation in the trigeminal nociceptive system, which is involved in migraine pain.	Della Pietra <i>et al.</i> (2023)
In trigeminal neurons			Piezo2 channels are found expressed in trigeminal ganglion and corneal neurons, suggesting they might transduce noxious mechanical stimuli. Bron <i>et al.</i> (2014)
In trigeminal neurons			Piezo2 channels in corneal neurons are part of a polymodal nociceptive profile. Alamri <i>et al.</i> (2015)
In trigeminal neurons			Piezo2 were found responsible for transmitting low intensity yet harmful mechanical forces, resulting in ocular discomfort and triggering protective blink reflexes. Fernández-Trillo <i>et al.</i> (2020)
In trigeminal neurons			Expression of Piezo2 channels in trigeminal ganglion leads to dental pain and oral sensory perception. Emrick <i>et al.</i> (2020)
In trigeminal neurons			Co-expression of Piezo2 with other receptors in dental primary afferent neurons might suggest that they have a unique sensory population of trigeminal neurons. Lee <i>et al.</i> (2020)
In trigeminal neurons		Functional expression of Piezo1 in dorsal root ganglion (DRG) neurons is considered to participate in mechanical hypersensitivity and nociception. Moreover, Piezo1 channels can be negatively regulated by the co-expressed TRPV1 channels in mouse DRG neurons.	Roh <i>et al.</i> (2020)
In trigeminal neurons		Piezo1 channels are expressed in odontoblasts and it was found that inward currents recorded from medium-sized trigeminal neurons, were significantly inhibited by antagonists for the Piezo1 channel. This implies that Piezo1 is involved in transducing mechanical stimuli into electrical signals, as part of the sensory response mechanism.	Sato <i>et al.</i> (2018)

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Table 1. Continued from previous page.

Role of Piezo1 and Piezo2	Role of Piezo1	Role of Piezo2
In trigeminal neurons	The expression and activation of Piezo1 channels in odontoblast-like cells (OLCs) and dental pulp cells highlights their contribution in dental hypersensitivity, proposing their involvement in the release of ATP, the generation of slow inward currents, and the increase in action potentials in trigeminal ganglion neurons leading to transmission of pain signals.	Sun <i>et al.</i> (2022)
In trigeminal neurons	Piezo1 channels are predominantly expressed in small, myelinated axons known as A δ fibers in the dental pulp. Piezo1 channels in these locations play a crucial role in converting mechanical stimuli into electrical signals, contributing to the initiation of pain responses, particularly in the context of acute pain in dental pulp. The axons in the peripheral pulp and the dentinal tubules are the primary sites where Piezo1-mediated mechanotransduction is initiated.	Cho <i>et al.</i> (2022)
In trigeminal neurons		Piezo2 channels found to be regulated by IL-6 and showed increased expression following trigeminal nerve lesion, suggesting their involvement in neuralgia. Liu <i>et al.</i> (2021)
In trigeminal neurons		After a nerve compression injury, Piezo2 and ATP levels were found elevated underlying the contribution of ATP in Piezo2-mediated pain. Luo <i>et al.</i> (2022)
In trigeminal neurons		Piezo2 pronociceptive gene shows upregulation during masseter inflammation, highlighting the significance of DNA methylation as an epigenetic modulator of the Piezo2 gene. Abnormal DNA methylation process is a potential contributor to pathological pain responses in the orofacial region. Bai <i>et al.</i> (2020)
In Trigeminal neurons		Piezo2 as a novel contributor to transcriptional modifications in various nociceptor genes, indicating its potential role in pain signaling in the context of inflammation. Chung <i>et al.</i> (2016)

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Table 1. Continued from previous page.

	Role of Piezo1 and Piezo2	Role of Piezo1	Role of Piezo2
In trigeminal neurons		Piezo1 plays a role in trigeminal post-stroke headaches. Alongside TRPV1 channels, Piezo1 is implicated in the activation of trigeminal meningeal afferents following a photothrombotic stroke. Elevated expression of mechanosensitive Piezo1 channels, triggered by ischemic lesions and mechanical stimuli, contributes to nociceptive signals and the development of headaches.	Krivoshein <i>et al.</i> (2022)
In trigeminal neurons			Piezo2 is implicated in mechanosensory specialization within the trigeminal ganglia of tactile specialist birds. The growth of neuronal touch receptors containing Piezo2 occurs at the expense of receptors responsible for temperature and pain, indicating Piezo2's role in broader mechanosensory specialization mechanisms.

The role of piezo channels in migraine

We identified 5 original articles¹⁶⁻²⁰ that studied the relationship between Piezo channels and migraine pain.

Piezo receptors have been found to be involved in the generation of migraine pain as a result of meningeal nociception.¹⁶ Mikhailov *et al.*¹⁶ provided evidence demonstrating the presence of mechanosensitive Piezo1 and Piezo2 channels in rat and mouse trigeminal ganglia by combining diverse experimental approaches. It was proven that Piezo receptors activation has a pronociceptive effect on neuronal firing and stimulates the release of the migraine mediator calcitonin gene-related peptide (CGRP) in the somas of trigeminal neurons as well as in peripheral meningeal nerve fibers.¹⁶ These results imply that these mechanosensitive channels can cause pain to be generated in the meninges and can do so by increasing the sensitivity of nearby neurons to CGRP (Figure 5).¹⁶

Using an approved electrophysiological migraine model, Dolgorukova *et al.*¹⁷ showed distinct effects of low and doses of Yoda1, a Piezo1 agonist, on the central trigeminovascular neurons *in vivo*, laying the groundwork for future studies on mechanotransduction in migraine pathogenesis. They also demonstrated the vasodilator effect of Yoda1 on the arteries innervated by trigeminal afferents using intravital microscopy, indicating that Piezo1 may play a role in controlling vascular tone during a migraine attack,¹⁷ a vital component in migraine pathophysiology.¹⁰

In addition, Krivoshein *et al.*¹⁸ studied the migraine-relevant sex dependent activation of meningeal afferents in mice. An important finding regarding Piezo1 channel activation, was the lack of sex dependance in mice, leaving these channels out of the equation for the female prevalence of migraine.¹⁸

To address the specificity of the mechanosensitivity of trigem-

inal *versus* somatic nerves in migraine, Piezo1 channels were evaluated in mouse trigeminal *versus* DRG neurons by using a standard live calcium imaging setup with a multibarrel application system and a microfluidic chip-based setup.¹⁹ Surprisingly, it was found that trigeminal neurons, despite having higher Piezo1 gene expression, had less functional Piezo1 receptors than the DRG neurons.¹⁹ In fact, they showed lower sensitivity to the Piezo1 agonist Yoda1. However, because trigeminal neurons express more Piezo-coding mRNA than DRG neurons, and concurrently both types of neurons express the protein at roughly similar levels, trigeminal neurons appear to have a reserve capacity that can quickly increase Piezo1 functionality, when necessary, *e.g.*, during a migraine attack.¹⁹

Satellite glial cells (SGCs) were used as a novel method to detect Piezo1 channel activity in mouse trigeminal neurons, by utilizing live calcium imaging with Fluo-4 AM and labeling with FM1-43 dye.²⁰ This can track both current and past Piezo1 channel activation in the trigeminal nociceptive system, offering further evidence for Piezo1 implication in migraine.²⁰

The role of Piezo channels in trigeminal neurons

We identified 15 original research papers²¹⁻³⁵ that studied the role of Piezo1 and Piezo2 along with other nociceptors in trigeminal neurons. The papers were referring to various pain pathways involved in eye, dental and orofacial pain conditions.

Exploring the intricacies of corneal nociception, Bron *et al.*²¹ discovered that 26% of trigeminal ganglion neurons and 30% of corneal afferent neurons express Piezo2, revealing a distinct subset of mechano-nociceptors in the cornea. This sets the stage for an intriguing exploration of Piezo2's role in transducing noxious mechanical stimuli in an *in vivo* system.²¹ Alamri *et al.*²² continued

this investigation within the trigeminal ganglion, finding that around 45% of corneal afferent neurons also express Transient Receptor Potential Vanilloid 1 (TRPV1) channels suggesting a polymodal nociceptive profile which includes both Piezo2 and TRPV1, an observation that added complexity to the sensory landscape in corneal neurons.²² Also in humans, it has been shown that there is a clear involvement of Piezo2 channels in mechanosensory trigeminal neurons in the cornea.²³ This publication not only confirmed Piezo2's expression in corneal nociceptors but also demonstrated its role in transmitting mechanical forces, contributing to low-threshold mechanically evoked ocular discomfort and pain.²³ Additionally, Piezo2 in corneal neurons plays a pivotal role in recognizing low-intensity yet harmful mechanical forces, triggering protective blink reflexes.²³ This cohesive narrative underscores the multifaceted role of Piezo2 in corneal nociception, from its expression patterns to its involvement in mechanical force transmission and ocular discomfort.²¹⁻²³

Passing to the exploration of mechanisms underlying dental pain, Piezo2 have been proven to be expressed in a significant proportion of sensory neurons, including nociceptors, in the trigeminal ganglion responsible for dental innervation.²⁴ This suggests that Piezo2 may have a role in the mechanosensitive functions of these neurons, enabling them to respond to gentle touch stimuli in addition to their nociceptive functions, which could be relevant in understanding the sensation of dental pain and oral sensory perception.²⁴

Additionally to the existing dental pain research, it has been

shown that Transient Receptor Potential Cation Channel Subfamily M Member 8 (TRPM8) receptors expressed in dental primary afferent neurons may act as a hyperosmosensors in adult mice to mediate dental pain.²⁵ The preferential coexpression of TRPM8 with TRPV1, CGRP, and Piezo2 in dental primary afferent neurons might suggest that they have a unique sensory population of trigeminal neurons distinct from DRG neurons.²⁵

Piezo1 were also proven to have physiological functions in DRG neurons, discovering that TRPV1 activation inhibits an inward current induced by the Yoda1, a Piezo1 agonist.²⁶ Mechanosensitive Piezo1/ Transient Receptor Potential (TRP) channels respond to hydrodynamic forces deforming odontoblasts. Adding to this, P2X purinergic receptors 3 (P2X3) on myelinated Aδ neurons are subsequently activated and this induces adenosine triphosphate (ATP) release.²⁷ P2X3 receptor activation causes Aδ neurons to go into action potential, which causes dental pain. According to the "odontoblast hydrodynamic receptor theory", this neurotransmission from odontoblasts to trigeminal ganglion neurons explains the sensory mechanism that causes dental pain in a manner that is both simple and unambiguous.²⁷

Furthermore, *Sun et al.*²⁸ propose that odontoblasts, serving as sensory cells, transmit signals to adjacent cells and trigeminal neurons, generating pain responses. The expression and activation of Piezo1 channels in odontoblast-like cells (OLCs) and dental pulp cells play a crucial role.²⁸ *In vitro* experiments indicate that Piezo1 ion channels in OLCs conduct mechanical transduction,

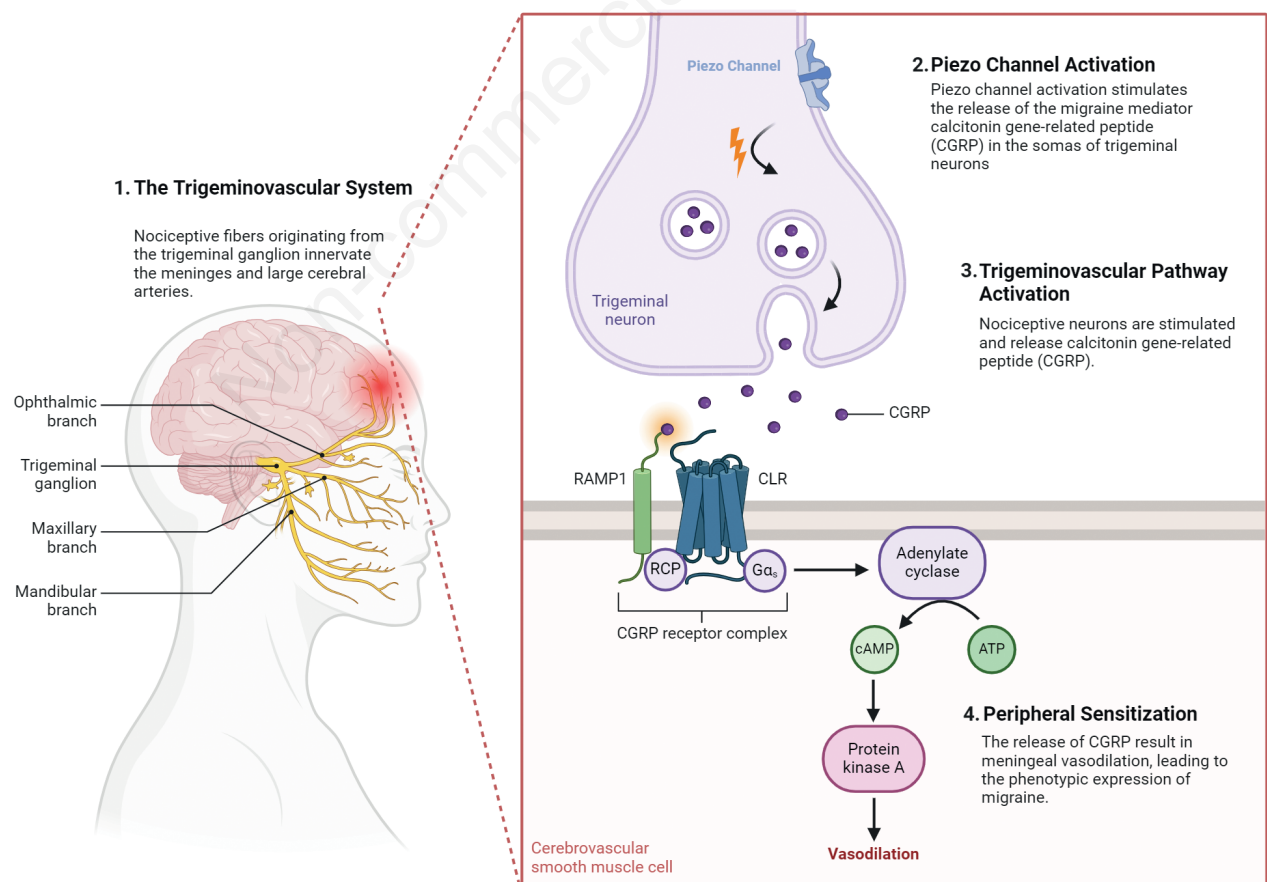


Figure 5. The role of piezo channels, CGRP, and the trigeminal system in migraine pathophysiology (created with BioRender.com).

resulting in the release of ATP, slow inward currents, and increased action potentials in trigeminal ganglion neurons.²⁸ This highlights the pivotal role of Piezo1 in transmitting pain signals, leading to dental hypersensitivity.²⁸

Investigating the expression of mechanosensitive Piezo1 channels, Cho *et al.*²⁹ focused on trigeminal neurons and axons in the sensory root of the trigeminal ganglion, as well as axons innervating dental pulp in rat and human models. Their findings proved that Piezo1 channels are mostly found in small, myelinated axons (A δ), proposing their contribution to mediation of acute pain.²⁹ Moreover, it was established that Piezo1 positive myelinated axons in the radicular pulp become unmyelinated in the peripheral pulp and form a network leading to the dentinal tubules, suggesting that the axons in the peripheral pulp and the dentinal tubules are the main sites of initiation of Piezo1-mediated mechano-transduction.²⁹

Passing from dental to orofacial pain, some of the included studies on trigeminal neurons, examined the role of Piezo channels in trigeminal neuralgia (TN).^{30,31} Liu *et al.*³⁰ propose a pivotal role for Piezo2 channels and IL-6 in trigeminal neuropathic pain. In rat models, Piezo2 channels showed increased expression following trigeminal nerve lesion, suggesting their involvement in trigeminal neuralgia.³⁰ The study also suggests a regulatory role of IL-6 in Piezo2 channel expression.³⁰ Expanding on this, Luo *et al.*³¹ investigated Piezo2 channel-mediated mechano-transduction in rat models of TN and nerve compression injury. They found elevated ATP receptors and increased Piezo2 expression, indicating a role in mechanical allodynia.³¹ The study underscores the contribution of extracellular ATP and cyclic adenosine monophosphate (cAMP) signaling pathways to Piezo2-dependent pain in trigeminal nerve compression injury.³¹

In a different context, Bai *et al.*³² conducted a study on rats' trigeminal ganglia, proposing that peripheral inflammation could induce alterations in the methylation patterns of certain pro-nociceptive genes, including Piezo2. Their experiments revealed that masseter inflammation led to decreased DNA methylation in trigeminal ganglia, concurrently reducing the expression of DNA methyltransferase (DNMT) enzymes.³² This decrease in enzyme levels resulted in the upregulation of various pronociceptive genes, highlighting the significance of DNA methylation as an epigenetic modulator of the Piezo2 gene.³² This abnormal DNA methylation process emerged as a potential contributor to pathological pain responses in the orofacial region.³² The findings underscore the complex interplay between inflammation, epigenetic modifications, and the expression of nociceptive genes in the context of trigeminal ganglia.³²

Chung *et al.*³³ performed a transcriptome analysis of trigeminal ganglia in rats with masseter muscle inflammation. They identified transcriptional modifications in various nociceptor genes, such as TRPV1 and Transient Receptor Potential cation channel, subfamily A, member 1 (TRPA1), associated with masseter hyperalgesia and they also uncovered Piezo2 as a novel contributor to these modifications, indicating its potential role in pain signaling.³³ Transitioning from inflammation to neurological events, Krivoshein *et al.*³⁴ investigated trigeminal meningeal afferents' activation after a photothrombotic stroke in mice and showed that Piezo1 along with TRPV1 channels do have an involvement in this pain pathway. Elevated expression of mechanosensitive Piezo1 channels and prolonged activation of nociceptive TRPV1 channels in ischemic hemispheres indicated a co-activation mechanism triggered by both ischemic lesions and mechanical stimuli, generating nociceptive signals leading to trigeminal post-stroke headaches.³⁴

All in all, Schneider *et al.*³⁵ conducted a cross-species analysis and revealed the hidden role of Piezo2 in mechanosensory specialization of trigeminal ganglia in tactile specialist birds. The research showed a trade-off in the growth of neuronal touch receptors containing Piezo2.³⁵ This trade-off occurs at the cost of receptors responsible for temperature and pain, forming part of a broader and Piezo2-related mechanism associated with mechanosensory specialization of trigeminal ganglia.³⁵

Convergence of mechanisms: Piezo channels, migraine, and trigeminal neurons

The interplay between Piezo channels, migraine, and trigeminal neurons uncovers a web of intricate associations and interaction and reveals potential insights into how Piezo channels influence migraine-related pain pathways and other trigeminal pain syndromes.

Beginning with the study of Mikhailov *et al.*, it is demonstrated that activation of Piezo channels in peripheral trigeminal ganglia generates migraine pain.¹⁶ It was formerly known that pain in migraine starts from the trigeminovascular system in meninges,³⁶ however this study proved through extensive experiments the pro-nociceptive role of the mechanosensitive Piezo channels in trigeminal fibers.¹⁶ It was found that activation of Piezo channels in rat and mice trigeminal ganglia led to meningeal nociception and prolonged pain generation due to the release of migraine mediator CGRP.¹⁶ This association also addresses the pulsating nature of migraine nociception, as it proposed that pain in migraine originates from regular pulsation-induced stimulations of Piezo channels found in sensory nerve fibers.¹⁶

Dolgorukova *et al.*'s study¹⁷ further reinforces the connection between trigeminal neurons and migraine. It emphasizes that migraine cannot occur without the activation of trigeminal neurons innervating the meninges.¹⁷ The activation of meningeal afferents releases substances like CGRP, enhancing neuronal excitability and contributing to pain during migraine attacks.¹⁷ The role of Piezo1 in migraine pathogenesis is investigated, suggesting that it may contribute to the activation of both vascular and neuronal components of the trigeminovascular system, resulting in migraine pain.¹⁷ The study underscores the differential effects of low and high doses of the Piezo1 agonist Yoda1 on central trigeminovascular neurons, offering a basis for further research on mechano-transduction in migraine pathology.¹⁷

Krivoshein *et al.*¹⁸ propose that sex hormones can activate trigeminal neurons innervating the meninges, leading to migraines. They suggest a direct relationship between trigeminal neurons, migraine pain, and sex.¹⁸ Although the study did not show any sex difference in the Piezo1 channel activation in meningeal afferents, it revealed a functional role of TRPM3 channels in nociception triggered by female sex hormones.¹⁸

Mikhailov *et al.*¹⁹ researched the mechanosensitivity of trigeminal *versus* somatic nerves, comparing the activity of Piezo1 receptors in trigeminal neurons and DRG neurons. Despite higher Piezo1 gene expression in trigeminal neurons, they were less responsive to the Piezo1 agonist Yoda1 compared to DRG neurons.¹⁹ This suggests that trigeminal neurons may have fewer active Piezo1 channels, a relevant consideration in migraine pathogenesis.¹⁹

Finally, current research by Della Pietra *et al.*²⁰ introduces a novel perspective on the role of Piezo1 channels within the trigeminal system, suggesting their involvement in migraine pain. Their experiments reveal the presence of Piezo1 channels



in both trigeminal neurons and SGCs, correlating extended activation of these channels with migraine pain.^{20]}This novel insight lays the foundation for potential cross-associations between Piezo channels, migraine, and trigeminal neurons, emphasizing the complex web of interactions in migraine pathophysiology.²⁰

Therapeutic implications and future directions

This section examines the therapeutic implications and future directions in the rapidly evolving field of Piezo channels, migraine, and trigeminal neurons.

The included studies of this systematic review, and the latest understanding of mechano-transduction in migraine pathogenesis revealed that Piezo1 receptors should be considered as one of the molecular targets in migraine treatment.¹⁶ Satellite glial cells (SGCs) surrounding the bodies of trigeminal neurons are an additional new target for suppressing the pro-nociceptive activity of Piezo channels in the trigeminovascular system, as evidenced by the detection of functional Piezo1 activity in trigeminal SGCs.²⁰

Additionally, the correlation between Piezo channels and trigeminal neurons introduces novel ideas on possible therapeutic targets for pain conditions related with this particular type of neurons. Research on trigeminal ganglia proved that DNA methylation process is an epigenetic modulator of the Piezo2 gene and changes in that process could lead to pathological pain responses.³² Subsequently, Bai *et al.*³² propose that future research on the treatment of chronic pain syndromes should focus on resolving the matter of DNA hypomethylation which possibly helps alleviate inflammatory pain. Fernández-Trillo *et al.*²³ examined the expression of Piezo2 in corneal trigeminal neurons and its role in pain transmission. In this study, it is suggested that topical inhibition of Piezo2 in the cornea could relieve painful sensations and discomfort associated with dry eye and other ocular surface pathologies.³³ However, due to the vital role of Piezo2 in mechano-transduction in various organs, systematic inhibition of Piezo2 for pain relief is not an option.²³ Sun *et al.*²⁸ demonstrated that Piezo1 channels transmit mechanical stimuli in odontoblasts and trigger trigeminal neurons causing pain and proposed these channels as a possible therapeutic target for treating cases of dentin hypersensitivity.²⁸ Similarly, Cho *et al.*²⁹ also studied the role of Piezo1 channels in trigeminal neurons and axons innervating the dental pulp suggested local use of a Piezo1 channel blocker in the peripheral pulp or dentinal tubule in order to alleviate dentin sensitivity without the negative effects of a systemic administration.²⁹

Liu *et al.*³⁰ shed light on a new potential noninvasive targeting treatment of trigeminal neuralgia (TN) by blocking the IL-6/Piezo2 pathway. Their study showed that increased IL-6 levels upregulate the expression of Piezo and altogether modulate trigeminal neuropathic pain.³⁰ Blockage of these 2 molecules is a new proposed treatment plan and will come as an alternative to invasive brain surgery.³⁰ All in all, Krivoshein *et al.*³⁴ studied the involvement of Piezo1 channels in post-stroke headaches and proved that animal stroke models with Piezo1 channel suppression showed reduced infarct size and effective inhibition of arterial thrombosis.³⁴

All the above insights into mechano-transduction mechanisms provide new avenues for research and the development of targeted interventions, offering hope for more effective migraine management in the future.

Discussion

This systematic review evaluated the role of Piezo channels in migraine and their involvement in trigeminal neurons. Our review included a total of 20 studies,¹⁶⁻³⁵ published between 2014 and 2023. These studies shed light on the intricate relationship between Piezo channels, migraine pathophysiology, and trigeminal neurons, providing valuable insights into mechano-transduction processes and potential therapeutic avenues.

The studies focusing on the association between Piezo channels and migraine¹⁶⁻²⁰ collectively indicate that these mechanosensitive receptors may indeed contribute to migraine pain. Furthermore, the role of Piezo channels in trigeminal neurons emerged as a prominent theme in our review, with many of the included articles showing a strong relation.²¹⁻³⁵ Besides, the existing literature has underlined that mechanosensitive nerve endings that branch out from the trigeminal ganglia are activated, converting mechanical stimuli into action potentials that mediate numerous pain signals.^{7,37,38}

Despite the promising findings regarding Piezo channels in migraine and trigeminal neurons, it is important to note that only a limited number of studies suggested potential targets for treatment. Thus, future research should focus more on the evolving field of molecular pain, to elucidate the role of Piezo1 and Piezo2 in different types of pain.

Apart from Piezo channels, TRPM3 channels were also suggested as a promising target for pharmacological interventions in migraine, taking into account their molecular and mechanosensitive characteristics.¹⁸ According to the existing literature, CGRP has also been reported as a key migraine mediator^{39,40} and it is well known that CGRP sensitizes trigeminal neurons, causes migraine episodes, and has pro-nociceptive effects in migraine.^{41,42} Chronic migraine sufferers have higher CGRP levels,⁴³ and lowering CGRP levels can help alleviate migraines.⁴⁴

The knowledge around Piezo channels' pharmacology is extremely inadequate despite their substantial significance in physiology and their potential roles in numerous diseases.⁴⁵ Both gain and loss of function in Piezo mutations are known to cause disease, such as somatosensory and proprioceptive disorders, or blood discrepancies.⁴⁵ As a result, pharmacological inhibitors and activators for Piezo1 and Piezo2 channels should be a future research goal.⁴⁵

There are several strengths of this review that are important to highlight. To our knowledge, this is the first systematic review for Piezo channels in migraine and other pain conditions implicated with the trigeminal neurons, providing a fresh perspective that may challenge existing theories and inspire new research directions. The review was performed according to a pre-registered protocol (CRD42023481700). To give a thorough review of the literature in this field, we also included articles from a variety of sources. Finally, we performed quality assessment of the included animal studies to ensure that there was no high risk of bias.

However, our review was not without its limitations. Given that this is a relatively under-investigated field, only a limited number of studies were included into our review. Piezo1 and Piezo2 channels are still the early days of their exploration, therefore the studies evaluated in this review were mainly animal-based, highlighting the urgent need for further research involving human subjects. We tried to differentiate the articles referring to migraine from the articles referring to the trigeminal neurons, although we understand that the complex pathophysiology of the former includes mechanisms through the trigeminal neurons. Larger samples as



well as clinical applications of these experiments is needed to completely examine the true relationship between Piezo channels and pain and at the same time their role as therapeutic targets.

Conclusions

The mechanistic insights derived from this systematic review underscore the need for a comprehensive exploration of mechano-transduction processes in migraine and numerous other pain conditions involving trigeminal neurons. Piezo channels, as well as other nociceptors, collectively contribute to the intricate web of migraine pathophysiology. Understanding their roles within the context of mechano-transduction may hold the key to unraveling the mysteries of migraine and trigeminal-associated pain, potentially leading to more targeted and effective therapeutic interventions in the future.

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