Epigenetic connections of the TRPA1 ion channel in pain transmission and neurogenic inflammation – a therapeutic perspective in migraine?

Michal Fila[1], Janusz Blasiak[2*], Elzbieta Pawlowska[3]

[1]Department of Developmental Neurology and Epileptology, Polish Mother's Memorial Hospital Research Institute, 93-338 Lodz, Poland
[2]Department of Molecular Genetics, Faculty of Biology and Environmental Protection, University of Lodz, 90-236 Lodz, Poland
[3]Department of Pediatric Dentistry, Medical University of Lodz, 92-217 Lodz, Poland
* Correspondence: janusz.blasiak@biol.uni.lodz.pl; Tel.: +48-426354334

Abstract

Background: Transient receptor potential cation channel subfamily A member 1 (TRPA1) is expressed in trigeminal neurons and brain regions important in migraine pathogenesis and is activated by many migraine triggers. Epigenetic regulation of *TRPA1* expression is important in pain transmission and neurogenic inflammation.

Findings: TRPA1 channels change noxious stimuli into pain signals with the involvement of epigenetic regulation, including DNA methylation, histone modifications, and effects of micro RNAs (miRNAs) and long non-coding RNAs. TRPA1 may change epigenetic profile of many pain-related genes as it may modify enzymes establishing the epigenetic profile and expression of non-coding RNAs. TRPA1 may induce the release of calcitonin gene related peptide (CGRP), from trigeminal neurons and dural tissue. Therefore, epigenetic regulation of *TRPA1* may play a role in efficacy and safety of anti-migraine therapies targeting TRP channels and CGRP. TRPA1 is also involved in neurogenic inflammation, important in migraine. The fundamental role of TRPA1 in inflammatory pain transmission may be epigenetically regulated.

Conclusions: Epigenetic connections of *TRPA1* may play a role in efficacy and safety of anti-migraine therapy targeting TRP channels or CGRP and they should be further explored for efficient and safe antimigraine treatment.

Keywords: epigenetics; TRP channels; TRPA1; pain transmission; neuropathic pain; neurogenic inflammation; migraine; DNA methylation; histone modification; micro RNA

Introduction

Targeting calcitonin gene-related peptide (CGRP) and its receptor has opened a new era in migraine therapy, but this disorder is still undertreated. One of the main reasons for that is incompletely known mechanisms of its pathogenesis and consequently – poorly recognized molecular mechanisms of the action of accepted and perspective anti-migraine drugs.

The regulation of the expression of the calcitonin related polypeptide alpha (*CALCA*) gene, encoding an isoform of CGRP expressed in trigeminal ganglia and playing an import role in pain transmission, arouses a significant interest to improve migraine therapy targeting CGRP or its receptor. We recently provided some arguments that epigenetic regulation of the *CALCA* gene may be explored to increase efficacy and safety of migraine treatment [1].

Transient receptor potential cation channel subfamily A member 1 (TRPA1, TRP ankyrin 1), also known as the Wasabi receptor, is a member of TRP channels superfamily of ion channels. TRPA1, along with transient receptor potential cation channel subfamily V member 1 (TRPV1), TRPV4 and transient receptor potential cation channel subfamily M member 8 (TRPM8), is the most consistently reported to associate with migraine among all TRP channels [2]. These channels respond to stimuli involved in migraine pathogenesis and TRPA1 is reported to link stimulants of headache pain and migraine as well as mediate the vasodilatory response to headache-evoking environmental irritants [3, 4]. Also, TRPA1 is involved in CGRP release [5]. TRPA1, similarly to other TRP channels, is epigenetically regulated and may affect genes and proteins involved in the maintenance of the epigenetic profile [6, 7].

Association of TRP channels with CGRP release and migraine therapy, migraine as a channelopathy and an important role of epigenetic regulation in TRPA1 in pain transmission justify studies on the potential role of epigenetics in perspective TRPA1-targeting antimigraine drugs. The TRPA1 channel has likely provoked the most interest in preclinical headache and migraine studies among all TRP channels [2]. In this review we present and update information on the role of epigenetics in TRPA1 regulation and its interaction with other proteins and regulatory RNAs in pain transmission, neurogenic inflammation and pain-related syndromes with potential consequences for migraine pathogenesis. We also present arguments for the importance of epigenetic effects in a perspective TRPA1-targeting anti-migraine therapy.

TRPA1 – a member of transient receptor potential channel gene superfamily

Transient receptor potential cation channel subfamily A member 1 (TRPA1, TRP ankyrin 1), also known as the Wasabi receptor, is a member of TRP superfamily of ion channels, consisting of 28 members in mammals. They are categorized into six main families: TRP ankrin (TRPA), TRP canonical (TRPC), TRP melastatin (TRPM), TRP mucolipins (TRPML), TRP polycystin (TRPP), and TRP vanilloid (TRPV). TRP channels are tetrameric forms of nonselective Ca^{2+} influx channels. They have six transmembrane domains with both termini embedded in the cytosol. They are involved in environment perception and sense various stimuli, including visual, gustatory, olfactive, auditive, mechanical, thermal, and osmotic influences. Most, if not all, of these stimuli can be, directly or indirectly, related to migraine. Mutations in TRP channels are associated with various diseases and some of them have been classified as channelopathies ("TRPpathies"). These include some neurological disorders [8].

TRPA1 is encoded by the TRPA1 gene, which is located at 8q21.11, has 29 exons and 70,094 bp (GRCh38/hg38) and yields 3 transcripts translated to two 1119 aa and single 1144 aa proteins (https://www.ncbi.nlm.nih.gov/gene/8989, accessed October 03, 2022).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

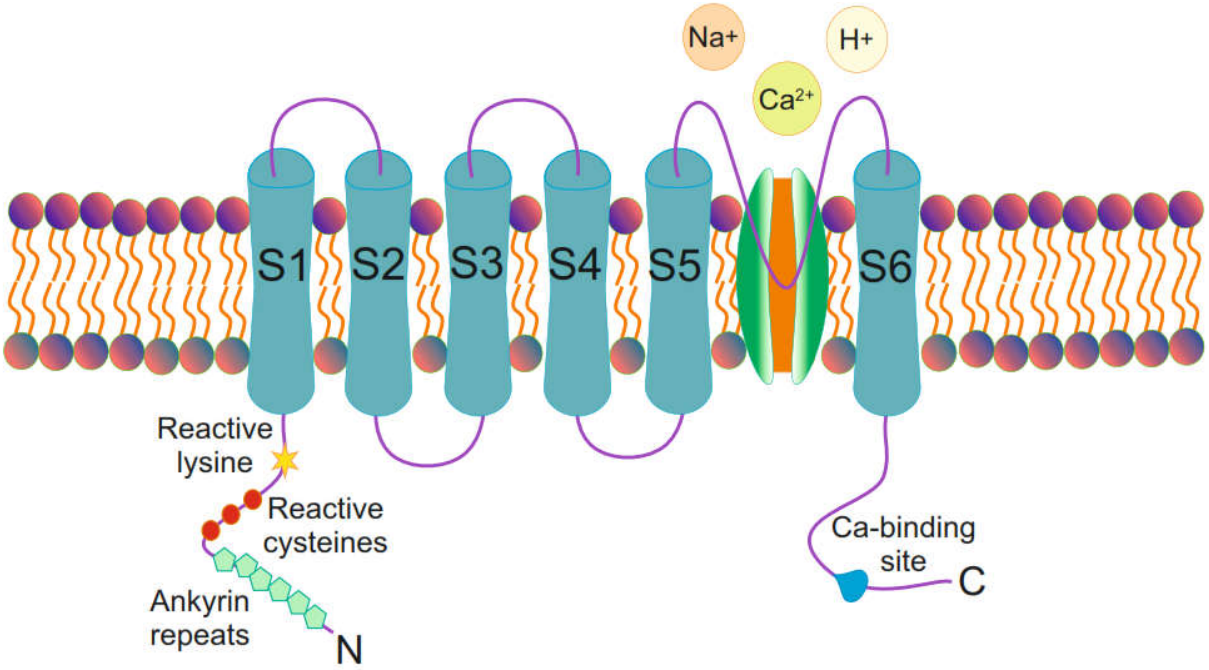

Figure 1. Structure of the transient receptor potential cation channel subfamily A member 1 (TRPA1) channel. TRPA1 contains 6 transmembrane domains (S1-S6) and its both termini (N and C) are embedded in the cytosol. The central pore region with selectivity filter for ions to enter and exit the cell membrane is situated between S5 and S6. The N-terminus contains ankyrin repeats and reactive cysteine residues that are involved in many functions of TRPA1. The calcium binding site is localized in the C-terminus.

TRPA1 belongs to the TRP ankyrin (TRPA) subfamily of the TRP channel superfamily. It is widely expressed in the cell surface of human cells, including neuronal cells [9]. TRPA1 is principally expressed on myelinated Aδ- and unmyelinated C-fibers of peripheral nerves. It is expressed on cell bodies of dorsal root ganglion, nodose ganglia, and trigeminal ganglia neurons, as well as on the axons of spinal nerves, the vagus nerve, and trigeminal nerve [10]. The human TRPA1 has four subunits associating into a channel. Similar to the remaining TRP channels, each TRPA1 subunit has six transmembrane domains, S1-S6, with bundles at the periphery of the channel formed by S1-S4 and a pore region and selectivity cation filter between S5 and S6 (Figure 1). In its N-terminal region TRPA1 has a domain of 14-18 ankyrin repeats, 33 amino acids each. This domain is important for the connection of TRPA1 with cytoskeleton, TRPA1 trafficking and protein-protein interactions. The C621, C633 and C635 cysteines are essential for the TRPA1 response to reactive electrophiles and they are located in the membrane-proximal N-terminal region that surrounds the TRP-domain helix [11]. Channel opening is regulated by upper and lower gates formed by sidechains of asparagine 915 of pore helix 1 and hydrophobic S6 residues isoleucine 957 and valine 961, respectively, projected into the pore [11]. TRPA1 may also contain other, demonstrated and putative, functional handles. The opening and closing of the gates are allosterically controlled by TRPA1 agonists and antagonists.

TRPA1 in pain transmission and neurogenic inflammation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

TRPA1 activation in nociceptors evokes action potentials signaling pain and induces an aversive or protective response and malfunction of TRPA1 may result in impaired pain signaling [12].

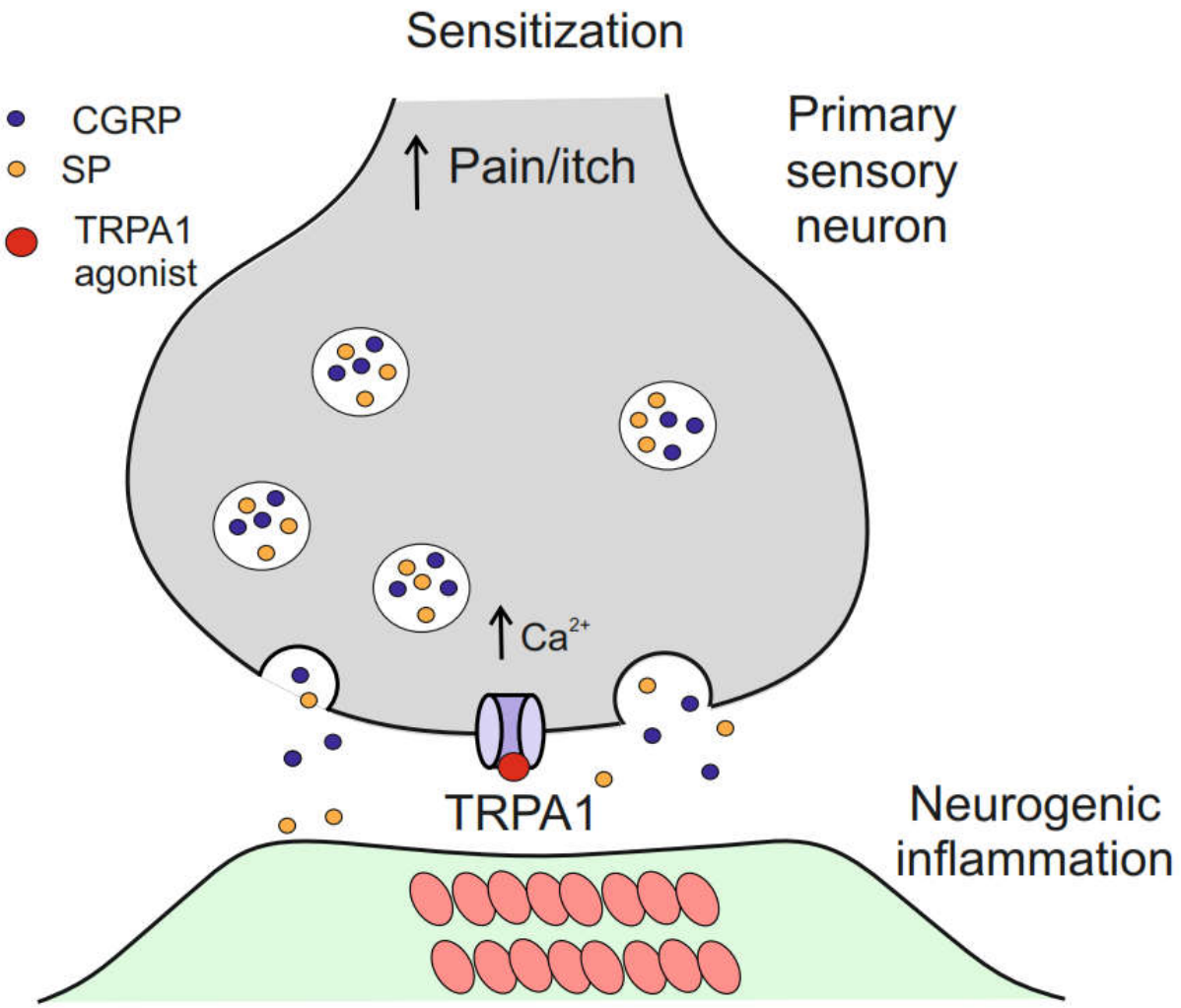

Figure. 2. Involvement of the transient receptor potential cation channel subfamily A member 1 (TRPA1) channel in the pain pathway. A TRPA1 agonist may desensitize the channel that is expressed on primary sensory neurons. Activation of TRPA1 by agonists induces an increased calcium influx, the release of pro-inflammatory neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P (SP), initiating the cascade of events resulting in neurogenic inflammation. At the same time, an impulse is generated that is sensed in the brain as pain or itching.

TRPA1 agonists, including reactive oxygen and nitrogen species, support the NMDA response and in this way amplify pain signaling from primary sensory neurons expressing TRPA1 on their surface to projection neurons (Figure 2) [12]. Activation of TRPA1 by agonists may lead to the release of neuropeptides, including CGRP and substance P (SP) that may generate neurogenic inflammation, an important aspect of migraine pathogenesis.

The activation of TRPA1 in rat dorsal root ganglion neurons increased its expression at mRNA and protein levels, as shown with the use of formalin and menthol that are TRPA1 agonist and antagonist,

respectively [5]. TRPA1 activation induced an increased expression of CGRP, which was inhibited by pretreatment with PD98059, an extracellular signal-regulated protein kinase 1/2 (ERK1/2) inhibitor, but it did not affect TRPA1 expression in the presence of formaldehyde or menthol. It could be concluded from that study that TRPA1 agonists/antagonists upregulate/downregulate the expression of both TRPA1 and CGRP and the ERK1/2 signaling pathway may mediate TRPA1-induced CGRP activation.

The involvement of TRPA1 in pain and inflammation may be partly underlined by endogenous TRPA1 agonists generated in oxidative stress or tissue injury, making it as a druggable target in pain-related disorders [13-16]. This is supported by many small molecular weight natural TRPA1 agonists and antagonists. A967079 and HC-030031 are pharmacological antagonists of TRPA1 shown to reduce peripheral neuropathy-associated pain and hypersensitivity in mice [17-20]. High resolution examination of the TRPA1 structure revealed a ligand-binding pocket in a TRP channel that can be targeted by drugs [11].

As thermo-TRPs are expressed in C and Aδ-fibre sensory neurons, which are involved in the transmission of pain signals, it was suggested that TRPA1, contributed to transmission and modulation of the nociceptive signals [21]. This was supported by the observation that TRPA1 agonists induce a range of reactions from a pleasant hot feeling to unpleasant pain, as in the case of allyl isothiocyanate, a component of mustard or wasabi [22]. Another support of the involvement of thermo-TRPs in the control of pain transmission is a decrease in intensity of some pain conditions by topical application of capsaicin, an selective agonist of TRP vanilloid 1 (TRPV1) [23]. TRPA1 stimulation was associated with an increased release of sensory neuropeptide from meninges and spinal dorsal root resulting in a neuroinflammatory state [10].

TRPA1 in migraine

Historically, it was TRPV1 and capsaicin that showed a potentially causative role of TRP channels in cluster headaches and migraine [24-26].

Many compounds, identified as TRPA1 agonists, including tobacco smoke, garlic, chlorine, formaldehyde and others, are known as migraine attack triggers [27-31]. TRPA1 is plentifully expressed in primary sensory neurons and is considered as sensors of chemical-, heat- and mechanical-induced pain, plying a major role in migraine pain [32]. As mentioned, TRPA1 induces the release of CGRP.

There are some key discoveries relating TRPA1 to migraine pathogenesis. It was observed that exposure to extract from *Umbellularia californica* tree ("the Headache Tree") provoked headache attacks [3]. Subsequently, it was shown that umbellulone stimulated TRPA1 and its stimulation in the dura by umbellulone and mustard oil induced headache responses in rats [33-35].

The effect of umbellulone, along with another TRPA1 agonist, mustard oil, on the dural-projecting rat trigeminal ganglion neurons were studied with whole-cell patch-clamp recordings in vitro [33]. Application of both agonists to dural afferents produced TRPA1-like currents in about 40% of cells. Dural application of both agonists in in vivo behavioral model of migraine-related allodynia induced robust time-related tactile facial and hind paw allodynia that was weakened by pretreatment with the TRPA1 antagonist HC-030031. This study showed that TRPA1 was expressed in a significant proportion of dural afferents, and activation of meningeal TRPA1 induced behaviors consistent with those observed in patients during migraine attacks. Furthermore, activation of meningeal TRPA1 could result in afferent signaling and headache.

Nitroglycerine (NTG), a nitric oxide (NO) donor drug, is commonly used to induce migraine in experimental studies. NO was reported to target TRPA1 and nitrosylate its cysteine residues with a significant selectivity over other NO-sensitive TRP channels [16]. This process may lead to the channel

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

sensitization and potentiation of CGRP release by other agents, resulting in increased neuroinflammation and pain response [21].

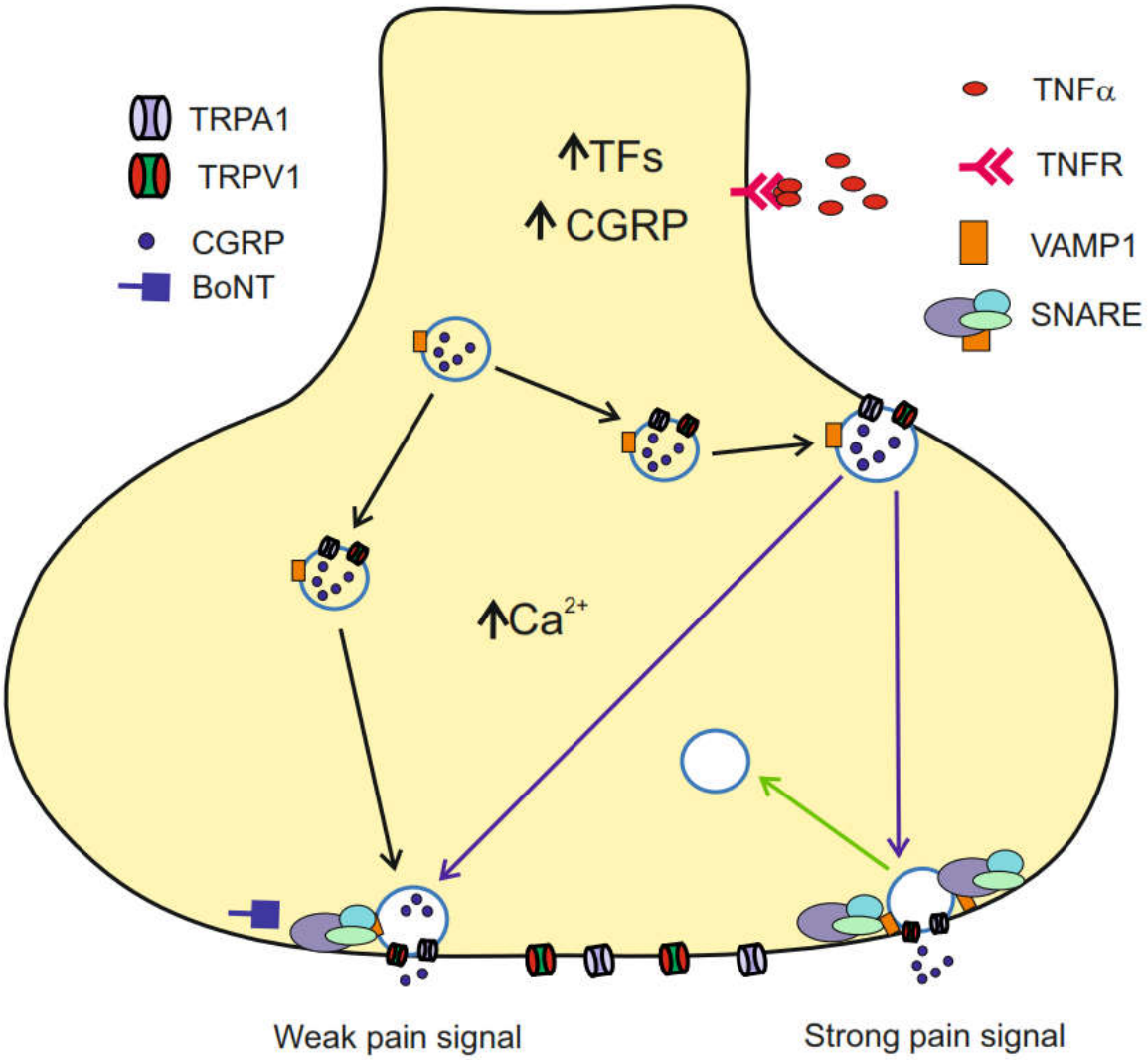

Figure 3. Tumor necrosis factor alpha (TNFα) induces pain-related effects in sensory neurons. TNFα may be released by inflammatory cells during pain induction and binds its receptor (TNFR). This results in an increase in calcium ion concentration and the stimulation of several translational factors (TFs) to increase calcitonin gene-related peptide (CGRP) release, which is at a low level in normal conditions. These events lead to enhanced trafficking of vehicles containing CGRP, transient receptor potential cation channel subfamily A member 1 (TRPA1) and transient receptor potential cation channel subfamily V member 1 (TRPV1) and eventually to release CGRP and other pain-mediators. TRPA1 and TRPV1 are loaded on vehicles containing CGRP and vesicle associated membrane protein 1 (VAMP1) and delivered to the plasma membrane with the formation of the SNARE complexes. Onabotulinum toxin-A (BoNTA) may affect the SNARE complex preventing exocytosis and delivery of TRPA1 and other TRP channels to the neuronal surface, resulting in a diminished CGRP release and pain intensity.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

Onabotulinum toxin-A (BoNTA) has been approved for the prevention of headache in chronic migraine and recent clinical evidence has shown that it is effective in the prevention of high-frequency episodic migraine [36, 37]. Benemei and Dussor pointed at a role of an interplay between BoNTA, CGRP and TRP channels in migraine pathogenesis, concluding that BoNTA might decrease the release of CGRP due to TRP-related mechanisms [2]. One of such mechanisms may include an association between CGRP release and the inflammatory response to tumor necrosis factor alpha (TNFα) (Figure 3). It was shown that TRPA1 and TRPV1 were present on vesicles containing CGRP and colocalized on the fibres and cell bodies of cultured sensory neurons [38]. TNFα enhanced the surface content of both channels and induced their co-trafficking with the involvement of a synaptic vesicle associated membrane protein (VAMP1), essential for CGRP exocytosis. VAMP1 is a component of the SNARE complex also containing synaptosome associated protein 25 (SNAP25), syntaxin 1A (STX1A), and syntaxin binding protein 1 (STXBP1). Furthermore, BoNTA may inhibit elevated delivery of TNFα. It can be speculated that similar processes may occur when only TRPA1 or another TRP channel is singly present in some exosomes [38].

Activation and sensitization of the trigeminal primary afferent neurons innervating the dura and cerebral vessels is critical in migraine pathogenesis. It was shown that TRPA1-expressing neurons are clustered around a subset of dural afferent neurons, in contrary to TRPM8 channel [39]. It was concluded that TRPA1 channel contributed to the excitation of dural afferent neurons and the subsequent activation of the headache circuit, providing an anatomical basis for the functional significance of TRP channels in headache pathophysiology.

In a series of studies, Kunkler et al. showed that TRPA1 mediated meningeal vasodilation induced by environmental irritant that could induce sensitization of the trigeminal system and chronic migraine-like phenotypes in rats [40-42].

In summary, many migraine triggers are TRPA1 agonists. TRPA1 may be involved in the excitation of dural afferent neurons resulting in activation of headaches. Also, TRPA1 may be important in the mechanisms of action of BoNTA, an effective drug in the prevention of chronic and high frequency episodic migraine. NTG, a migraine-inducer, displays a high selectivity toward TRPA1 over other TRP channels.

Epigenetic connections of TRPA1 in pain transmission and neurogenic inflammation

DNA methylation

Pain sensitivity is a complex phenotype as it may be underlined by many biological, environmental/lifestyle and psychological factors, but their effects depend on individual pain susceptibility, which is approximately only in half determined by genetic constitution. This conclusion is supported by twin-based heritability, animal research and genetic association studies [43-47]. Pain related signals are amplified in biological processes resulting in a chronic pain. These processes include sensitization of the nociceptive nerve fibres innervating peripheral tissues and sensitization of the spinal circuits relaying signals associated with tissue damage [6]. Again, these effects are only partly determined by genetic constitution and the role of epigenetic mechanisms in these processes is emerging [48-50].

Bell et al. examined genome-wide DNA methylation at 5.2 million loci by methylated DNA immunoprecipitation and deep sequencing in peripheral blood leukocytes of 50 identical twins and 50 unrelated individuals to identify differentially methylated loci associated with high or low heat pain sensitivity [6]. Among identified nine differentially methylated DNA regions, the most pronounced difference showed the *TRPA1* gene. An increased expression levels of *TRPA1* in the skin at higher pain thresholds was

observed, in agreement with a downregulatory effect of DNA methylation in the *TRPA1* promoter. Therefore, the promoter of the *TRPA1* gene has a regulatory methylation region, affecting its expression and thermal sensitivity.

A genome-wide methylation analysis on monozygotic twins showed that methylation of a CpG dinucleotide in the promoter of the *TRPA1* gene was inversely associated with the threshold for heat-induced pain [51]. It was found that the CpG dinucleotides associated with heat-evoked pain were hypermethylated in individuals with a low threshold for pressure pain. Females displayed a higher DNA methylation extent combined with higher pressure pain sensitivities, as compared with males. This study supports the role of epigenetic regulation of TRPA1 in pain sensitivity. Such role was confirmed in studies showing a positive correlation between DNA methylation levels in CpG island in the *TRPA1* gene in human peripheral blood leukocytes and a number of neuropathic pain syndromes, which, in turn, were negatively correlated with mRNA *TRPA1* expression [7]. In a similar study, an increased rate of DNA methylation at –51 CpG in the promoter of the *TRPA1* gene was positively correlated with the Douleur Neuropathique Questionnaire score in chronic pain patients [52].

Crohn's disease is a type of chronic inflammatory bowel disease (IBD) associated with abdominal pain and involvement of sensory neurons innervating the gastrointestinal tract [53]. Activation of sensory neurons results in the release of neuropeptides, including CGRP and individuals with IBD are reported to suffer from migraine at a higher rate than those without IBD [54, 55]. 2,4,6-trinitrobenzene-sulfonic-acid (TNBS), a TRPA1 agonist, induced IBD-like symptoms in mice associated with the release of CGRP and SP neuropeptides [56]. These data suggest that TRPA1 may mechanistically link IBD with migraine. Therefore, epigenetic regulation of TRPA1 in IBD may have some common pathways with its counterpart in migraine.

TRPA1 was upregulated in colonic mucosa samples from Crohn's disease patients [57]. Gombert et al. showed that this increased *TRPA1* expression in Crohn's disease might be underlined by the disease-related changes in its epigenetic regulation [58]. They showed that the thresholds for pressure pain were lower in the patients than controls. Moreover, these thresholds were lower in females than males. Higher ratio of migraine in females is related to higher susceptibility to headache pain in women than men. Pain thresholds were negatively correlated with the CpG dinucleotide located 628 bp upstream from transcription start site in the promoter of the *TRPA1* gene. Again, that effect was more pronounced in female as compared to male patients. Furthermore, DNA methylation at the CpG in Crohn's disease patients increased with age, whereas it decreased in controls. Pressure pain thresholds increased with age in both cohorts.

Histone modifications

The involvement of TRPA1 in neurogenic inflammation, important in migraine pathogenesis, may be linked with the functioning of this protein in immune cells. Macrophage polarization refers to activation and orientation of macrophages in response to a stimulus. It was shown that mice with a double knockout in the *TRPA1* and apolipoprotein E (*APOE*) genes showed an increase in atherosclerosis plaques compared to mice with single knockout in the *APOE* gene after a high-fat diet treatment [59]. Atherosclerosis was linked with a significant alteration of macrophage polarization toward a proinflammatory phenotype. Activation of TRPA1 by cinnamaldehyde decreased atherosclerosis progression. The effect on macrophage polarization was associated with a TRPA1-mediated induction of trimethylation of lysine 27 in H3 histone (H3K27me3), resulting in a downregulation of M1 macrophage genes. This epigenetic modification of H3 histone is regulated by the polycomb repressive complex 2 (PRC2), specifically through enhancer of zeste

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

2 polycomb repressive complex 2 subunit (EZH2). TRPA1 protected EZH2 from the proteasome-dependent degradation, allowing PCR2 to induce the H3K27me3 modification. Therefore, TRPA1 epigenetically regulated H3K27 trimethylation level in macrophages and so it can be considered as both substrate for and inducer of epigenetic modifications.

High concentration of glucose in diabetes results in nerve damage and a vast majority of diabetic patients show diabetic neuropathy as a comorbidity and pain is the major symptom of this disorder [60]. Furthermore, although association between migraine and diabetes is not firmly established and requires further research, some data clearly support such an association [61]. In this context and the context of the role of epigenetic modifications of the *TRPA1* gene in pain transmission, the results obtained on the effect of histone acetylation on TRPA1 expression in diabetes may be important. Kong et al. showed that acetylation of H3 histone by histone acetyltransferase steroid receptor coactivator-1 (SRC1) at the *TRPA1* promoter might play a role in its expression and electrical activity of TRPA1 in diabetic rats [62]. That work focuses on diabetes-related effects and does not provide details on the possible role of such epigenetic modifications in pain transmission. Histone acetylation at the gene promoter locus is usually associated with an increased expression of that gene, but such change should be considered in the context of whole set of epigenetic modifications. Furthermore, functional changes of its product may not be related to this effect and require further research, especially in pathological conditions (diabetes). In general, the involvement of TRPA1 in transmission of pain induced by common disorders, as cancer or diabetes and the role of epigenetics in this involvement is worth further studies as in many cases, the most serious effects associated with a disease are these related to its secondary consequences. Type 2 diabetes mellitus is a good example of such state as its consequences, including neuro-, nephro- and retinopathy result in more harmful effects than diabetes per se [63].

Wang et al. presented a mechanism leading from pressure overload to heart failure with the involvement of TRPA1 stimulating calcium/calmodulin dependent protein kinase II gamma (CAMK2G, CaMKII), which inhibits histone deacetylase 4 (HDAC4) [64]. HDAC4 prevents effective DNA binding by myocyte enhancer factor 2A (MEF2A, MEF2), contributing to hypertrophic gene expression and heart failure.

miRNAs

MicroRNAs mainly exert their role through the interaction with mRNA or other regulatory ncRNA. However, miRNAs can be found in circulation where they are stable and have been identified as useful biomarkers for many diseases [65]. Quereshi et al. showed changes in the miRNA profile in serum in several rodent models of pain, including spinal nerve ligation and spared nerve injury models of neuropathic pain; a complete Freund's adjuvant (CFA) model of inflammatory pain; and a chemotherapy-induced model of pain [66]. Each model produced a unique miRNA expression profile, but with some common biological pathways in different models. All models showed alterations in the Wnt signaling pathway, which suggests that this pathway may be essential for pain pathogenesis. These studies showed usefulness of miRNAs in the circulation for pain-related studies and a perspective of their use in diagnosis and therapy of disorders associated with pain.

Unilateral spared nerve injury (SNI) model is used to investigate mechanisms of neuropathic pain induction [67]. It was shown that the expression of *Mus musculus* miR-449a (mmu-miR-449a) decreased in the SNI mice as compared with controls [68]. Transfection of dorsal root ganglion DRG neurons with that miRNA resulted in a decrease in *TRPA1* mRNA levels. This change occurred simultaneously with a decrease

 doi:10.20944/preprints202210.0083.v1

in mRNA calcium-activated potassium channel subunit α-1 (KCNMA1) and an increase in transmembrane phosphatase with tension homology (TPTE) in the DRG cells. However, a more detailed mechanism of possible interplay between TRPA1, KCNMA1 and TPTE mediated by mmu-miR-449a was not investigated and the only conclusion from that study was that mmu-miR-449a might decrease neuropathic pain through downregulation of TRPA1 and KCNMA1 and upregulation of TPTE. Consequently, mmu-miR-449a could be considered as a potential therapeutic target in neuropathic pain treatment.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

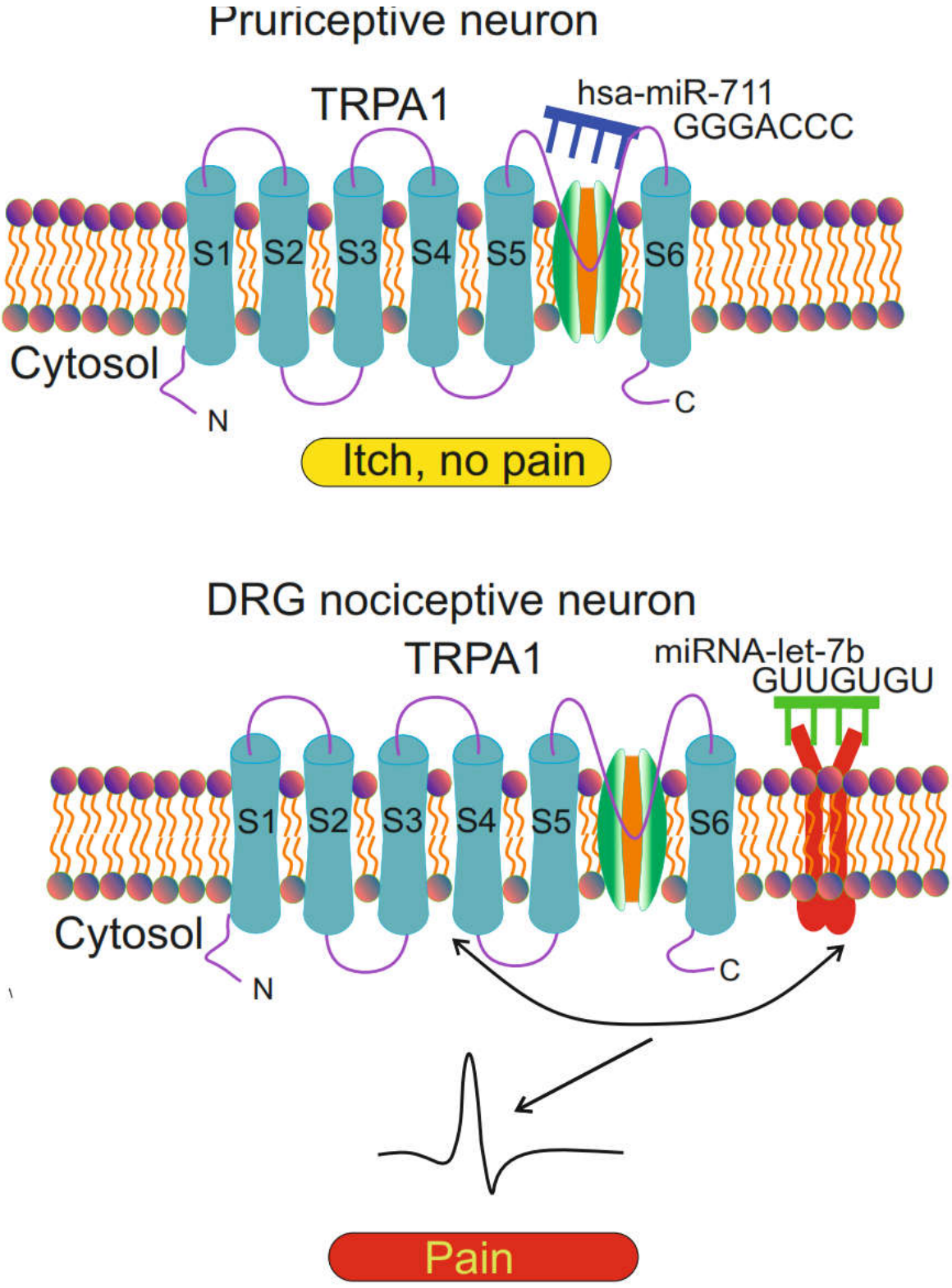

Figure 4. Role of microRNAs in the involvement of transient receptor potential cation channel subfamily A member 1 (TRPA1) in itch and pain dependent on and independent of toll-like receptor 7 (TLR7). In pruriceptive neurons, hsa-miR-711 binds TRPA1 in the extracellular S5-S6 loop with the core sequence 5'-GGGACCC-3' driving chronic or acute itch, but no pain. In dorsal root ganglion (DRG) nociceptive neurons,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 October 2022 doi:10.20944/preprints202210.0083.v1

miRNA-let-7b binds toll-like receptor 7 (TLR7) with its core sequence 5'-GUUGUGU-3', but only in cells expressing also TRPA1. The stimulation of the TLR7-TRPA1 interaction results in a fast inward current and action potential leading to pain.

Although itch and pain are on different sensory pathways, TRPA1 may be involved not only in the activation of nociceptive neurons but also in their pruriceptive counterparts as shown by Han et al. [69]. They demonstrated that hsa-miR-711, secreted by inoculated human lymphoma cells on the mouse back skin, bound TRPA1 extracellularly on pruriceptive neurons. This binding involved the core 5'-GGGACCC-3' sequence in the miRNA and the extracellular S5-S6 loop of TRPA1 to drive acute and chronic itch, but not pain (Figure 4). In contrary, TRPA1 stimulation by allyl isothiocyanate, resulted in pain and not itch. It is puzzling how TRPA1 is involved in so different sensory pathways as pain and itch. It was hypothesized that itch was induced by fast and transient activation of TRPA1 by hsa-miR-711 from the extracellular side. Slow and persistent TRPA1 activation from the intracellular side by allyl isothiocyanate resulted in pain. The relationship between neuropathic pain and neuropathic itch is poorly known and neuropathic itch, similarly to migraine, involves the activation of the trigeminal system and rash is associated with migraine in some studies [70-72].

The work of Han et al. seems to be in a sharp contrast with the work of Park et al. who showed the role of extracellular miRNAs in a rapid excitation of nociceptor neurons via toll like receptor 7 (TLR7) and its coupling to TRPA1 ion channel [73]. They demonstrated that miRNA-let-7b induced fast inward currents and action potentials in DRG neurons. That effect required the 5'-GUUGUGU-3' motif in miRNA-let-7 and occurred only in neurons that expressed TLR7 and TRPA1 and was abolished in mice with knockout in the *TLR7* or *TRPA1* gene. miRNA-let-7b induced TLR7/TRPA1-dependent single-channel activities in DRG neurons and HEK293 cells showing upregulated TLR7/TRPA1. Intraplanar injection of let-7b induced rapid pain via TLR7 and TRPA1. Lastly, miRNA-let-7b was released from DRG neurons by neuronal activation, and its inhibitor decreased formalin-induced TRPA1 currents and spontaneous pain. In conclusion, extracellular miRNAs may be pain mediators via TLR7/TRPA1 activation in nociceptor neurons. However, there is not any serious discrepancy between works of Han et al. and Park et al. The pain-unrelated effect observed by Han et al. was independent of TLR7 and included a direct binding to TRPA1, whereas Park et al. showed pain-related effects with the involvement of TLR7. Moreover, they used different miRNAs and the effect observed in either study cannot be generalized to all extracellular miRNAs, which was confirmed in the study of Han who showed that a mutated miRNA did not induce effects attributed to miRNA-711.

Winkler et al. suggested that TLR7-mediated divergent outcomes, such as neuronal apoptosis and pain stimulation in the central nervous system induced by extracellular miRNA-let-7b, might follow from different localization of TLR7 to the endosome in the cortical and hippocampal neurons or the plasma membrane in the sensory neurons [73-75]. Therefore, different types of neurons may traffic TLR7 to distinct locations in the plasma membrane, changing the functional response of neurons to stimulation by miRNA-let-7b. The general conclusion that can be drown from both work is that extracellular miRNAs can regulate neuronal functions by interaction with TRPA1 that can be TLR7-dependent or independent. miRNAs were also found in DRG nociceptors to regulate pain and sodium channels expression [76].

Conclusions and perspectives

Although we did not cite direct evidence in migraine, epigenetic modifications of the *TRPA1* gene were shown to play an important role in the generation and transmission of neuropathic pain. Generally,

migraine is not considered as a neuropathic pain syndrome, but there are many common mechanisms and proteins involved in pathogenesis of migraine and neuropathic pain. One of them are TLRs and effects on the neuroimmune interface, that justify considering a large extent of overlap between migraine and neuropathic pain [32, 77]. Allodynia, hyperalgesia, and expansion of nociceptive fields, associated with neuropathic pain, appear during most migraine attacks [78]. Migraine and neuropathic pain have some common core features: peripheral pain perception, peripheral sensitization at dorsal root ganglion or its cranial counterpart, including trigeminal ganglion and central sensitization at the spinal cord, brain stem nuclei and thalamus before definitive pain perception at the sensory cortical matrix [79].

Epigenetic modifications of TRPA1 were reported to play a role in other syndromes, including diabetes and diseases of the gastrointestinal tract that are reported to associate with migraine. Finally, epigenetic profile of the *TRPA1* gene as well as the interaction between circulating miRNA and *TRPA1* may be important in the release of CGRP, a key player in migraine pathogenesis.

Several works relate pain transmission with the DNA methylation level in the promoter of the *TRPA1* gene in peripheral blood. Also, studies on Crohn's disease dealt with TRPA1 in colonic mucosa and not in enteric neurons. Therefore, there is a problem to justify conclusions on the effect in the nervous system based on results obtained in the periphery. Epigenetic pattern is largely perpetuated from one generation to another, but it is modified during development and stem cells differentiation and this modification can be tissue- or organ-specific. However, it is difficult, if not impossible, to study epigenetic modifications in migraine target tissues in live humans, not only due to ethical objections, but also for technical constraints. Moreover, animal models of neuropathic pain seem to be more consistent than their migraine counterparts and in consequence more reliable molecular data can be obtained in neuropathic pain studies [80].

In conclusion, there are many premises to study epigenetic connections in pain-related syndromes, including migraine. Such studies may open new therapeutic modalities and improve existing ones in this largely untreated disease.

Article highlights

- TRPA1 is activated by many migraine triggers, involved in CGRP release
- TRPA1 is expressed in the brain regions involved in headache generation
- Despite TRPA1 agonists did not enter phase III of clinical trials, TRPA1 is still considered as a druggable therapeutic target
- Epigenetic modifications of the *TRPA1* gene are important in neuropathic pain and neurogenic inflammation
- TRPA1 may be epigenetically modified to assist anti-CGRP therapy and increase anti-migraine activity of its agonists

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials
Not applicable.

Competing interest
The authors declare no conflict of interest.

Funding
This research received no external funding.

ORCID iDs

Michal Fila 0000-0002-0748-7080
Janusz Blasiak 0000-0001-9539-9584
Elzbieta Pawlowska 0000-0002-5373-4783

Authors' contributions
Conceptualization, J.B., M.F.; writing—original draft preparation, J.B., A.J., E.P., and M.F.; writing—review and editing, J.B. All authors have read and agreed to the published version of the manuscript.

Acknowledgements
The authors thank Monika Kicinska for technical assistance.

References
1. Fila M, Sobczuk A, Pawlowska E, et al. Epigenetic Connection of the Calcitonin Gene-Related Peptide and Its Potential in Migraine. *International journal of molecular sciences* 2022; 23 20220530. DOI: 10.3390/ijms23116151.
2. Benemei S and Dussor G. TRP Channels and Migraine: Recent Developments and New Therapeutic Opportunities. *Pharmaceuticals (Basel)* 2019; 12 20190409. DOI: 10.3390/ph12020054.
3. Benemei S, Appendino G and Geppetti P. Pleasant natural scent with unpleasant effects: cluster headache-like attacks triggered by Umbellularia californica. *Cephalalgia : an international journal of headache* 2010; 30: 744-746. 20100201. DOI: 10.1111/j.1468-2982.2009.01988.x.
4. Nassini R, Materazzi S, Benemei S, et al. The TRPA1 channel in inflammatory and neuropathic pain and migraine. *Rev Physiol Biochem Pharmacol* 2014; 167: 1-43. DOI: 10.1007/112_2014_18.
5. Wang XL, Cui LW, Liu Z, et al. Effects of TRPA1 activation and inhibition on TRPA1 and CGRP expression in dorsal root ganglion neurons. *Neural Regen Res* 2019; 14: 140-148. DOI: 10.4103/1673-5374.243719.
6. Bell JT, Loomis AK, Butcher LM, et al. Differential methylation of the TRPA1 promoter in pain sensitivity. *Nat Commun* 2014; 5: 2978. DOI: 10.1038/ncomms3978.
7. Sukenaga N, Ikeda-Miyagawa Y, Tanada D, et al. Correlation Between DNA Methylation of TRPA1 and Chronic Pain States in Human Whole Blood Cells. *Pain Med* 2016; 17: 1906-1910. 20160205. DOI: 10.1093/pm/pnv088.
8. Zhao S and Rohacs T. The newest TRP channelopathy: Gain of function TRPM3 mutations cause epilepsy and intellectual disability. *Channels (Austin)* 2021; 15: 386-397. DOI: 10.1080/19336950.2021.1908781.

9. Zygmunt PM and Högestätt ED. TRPA1. *Handb Exp Pharmacol* 2014; 222: 583-630. DOI: 10.1007/978-3-642-54215-2_23.
10. Silverman HA, Chen A, Kravatz NL, et al. Involvement of Neural Transient Receptor Potential Channels in Peripheral Inflammation. *Front Immunol* 2020; 11: 590261. 20201023. DOI: 10.3389/fimmu.2020.590261.
11. Brewster MSJ and Gaudet R. How the TRPA1 receptor transmits painful stimuli: Inner workings revealed by electron cryomicroscopy. *BioEssays* 2015; 37: 1184-1192. https://doi.org/10.1002/bies.201500085. DOI: https://doi.org/10.1002/bies.201500085.
12. Koivisto A, Chapman H, Jalava N, et al. TRPA1: a transducer and amplifier of pain and inflammation. *Basic & clinical pharmacology & toxicology* 2014; 114: 50-55. 20131007. DOI: 10.1111/bcpt.12138.
13. Andersson DA, Gentry C, Moss S, et al. Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2008; 28: 2485-2494. DOI: 10.1523/jneurosci.5369-07.2008.
14. Gao S, Kaudimba KK, Guo S, et al. Transient Receptor Potential Ankyrin Type-1 Channels as a Potential Target for the Treatment of Cardiovascular Diseases. *Frontiers in physiology* 2020; 11: 836. 20200730. DOI: 10.3389/fphys.2020.00836.
15. Koivisto A-P, Belvisi MG, Gaudet R, et al. Advances in TRP channel drug discovery: from target validation to clinical studies. *Nature Reviews Drug Discovery* 2022; 21: 41-59. DOI: 10.1038/s41573-021-00268-4.
16. Nishida M, Kuwahara K, Kozai D, et al. TRP Channels: Their Function and Potentiality as Drug Targets. In: *Innovative Medicine* (eds Nakao K, Minato N and Uemoto S), Tokyo, 2015// 2015, pp.195-218. Springer Japan.
17. Chen J, Joshi SK, DiDomenico S, et al. Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation. *Pain* 2011; 152: 1165-1172. 20110312. DOI: 10.1016/j.pain.2011.01.049.
18. Eid SR, Crown ED, Moore EL, et al. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Mol Pain* 2008; 4: 48. 20081027. DOI: 10.1186/1744-8069-4-48.
19. Trevisan G, Materazzi S, Fusi C, et al. Novel therapeutic strategy to prevent chemotherapy-induced persistent sensory neuropathy by TRPA1 blockade. *Cancer research* 2013; 73: 3120-3131. 20130311. DOI: 10.1158/0008-5472.Can-12-4370.
20. Wei H, Hämäläinen MM, Saarnilehto M, et al. Attenuation of mechanical hypersensitivity by an antagonist of the TRPA1 ion channel in diabetic animals. *Anesthesiology* 2009; 111: 147-154. DOI: 10.1097/ALN.0b013e3181a1642b.
21. Benemei S, De Cesaris F, Fusi C, et al. TRPA1 and other TRP channels in migraine. *The journal of headache and pain* 2013; 14: 71. 20130813. DOI: 10.1186/1129-2377-14-71.
22. Andersen HH, Lo Vecchio S, Gazerani P, et al. Dose-response study of topical allyl isothiocyanate (mustard oil) as a human surrogate model of pain, hyperalgesia, and neurogenic inflammation. *Pain* 2017; 158: 1723-1732. DOI: 10.1097/j.pain.0000000000000979.
23. Derry S, Sven-Rice A, Cole P, et al. Topical capsaicin (high concentration) for chronic neuropathic pain in adults. *Cochrane Database Syst Rev* 2013: Cd007393. 20130228. DOI: 10.1002/14651858.CD007393.pub3.
24. Fusco BM, Barzoi G and Agrò F. Repeated intranasal capsaicin applications to treat chronic migraine. *Br J Anaesth* 2003; 90: 812. DOI: 10.1093/bja/aeg572.
25. Levy RL. Intranasal capsaicin for acute abortive treatment of migraine without aura. *Headache* 1995; 35: 277.

26. Sicuteri F, Fusco BM, Marabini S, et al. Beneficial effect of capsaicin application to the nasal mucosa in cluster headache. *Clin J Pain* 1989; 5: 49-53. DOI: 10.1097/00002508-198903000-00010.
27. Andrè E, Campi B, Materazzi S, et al. Cigarette smoke-induced neurogenic inflammation is mediated by alpha,beta-unsaturated aldehydes and the TRPA1 receptor in rodents. *The Journal of clinical investigation* 2008; 118: 2574-2582. DOI: 10.1172/jci34886.
28. Bautista DM, Movahed P, Hinman A, et al. Pungent products from garlic activate the sensory ion channel TRPA1. *Proceedings of the National Academy of Sciences of the United States of America* 2005; 102: 12248-12252. 20050815. DOI: 10.1073/pnas.0505356102.
29. Fujita F, Uchida K, Moriyama T, et al. Intracellular alkalization causes pain sensation through activation of TRPA1 in mice. *The Journal of clinical investigation* 2008; 118: 4049-4057. 20081113. DOI: 10.1172/jci35957.
30. Kesserwani H. Migraine Triggers: An Overview of the Pharmacology, Biochemistry, Atmospherics, and Their Effects on Neural Networks. *Cureus* 2021; 13: e14243. 20210401. DOI: 10.7759/cureus.14243.
31. McNamara CR, Mandel-Brehm J, Bautista DM, et al. TRPA1 mediates formalin-induced pain. *Proceedings of the National Academy of Sciences of the United States of America* 2007; 104: 13525-13530. 20070808. DOI: 10.1073/pnas.0705924104.
32. Iannone LF, De Logu F, Geppetti P, et al. The role of TRP ion channels in migraine and headache. *Neuroscience letters* 2022; 768: 136380. 20211130. DOI: 10.1016/j.neulet.2021.136380.
33. Edelmayer RM, Le LN, Yan J, et al. Activation of TRPA1 on dural afferents: a potential mechanism of headache pain. *Pain* 2012; 153: 1949-1958. 20120717. DOI: 10.1016/j.pain.2012.06.012.
34. Nassini R, Materazzi S, Vriens J, et al. The 'headache tree' via umbellulone and TRPA1 activates the trigeminovascular system. *Brain* 2012; 135: 376-390. 20111027. DOI: 10.1093/brain/awr272.
35. Zhong J, Minassi A, Prenen J, et al. Umbellulone modulates TRP channels. *Pflugers Arch* 2011; 462: 861-870. 20111029. DOI: 10.1007/s00424-011-1043-1.
36. Blumenfeld AM, Stark RJ, Freeman MC, et al. Long-term study of the efficacy and safety of OnabotulinumtoxinA for the prevention of chronic migraine: COMPEL study. *The journal of headache and pain* 2018; 19: 13. 20180205. DOI: 10.1186/s10194-018-0840-8.
37. Martinelli D, Arceri S, De Icco R, et al. BoNT-A efficacy in high frequency migraine: an open label, single arm, exploratory study applying the PREEMPT paradigm. *Cephalalgia : an international journal of headache* 2022; 42: 170-175. 20210818. DOI: 10.1177/03331024211034508.
38. Meng J, Wang J, Steinhoff M, et al. TNFα induces co-trafficking of TRPV1/TRPA1 in VAMP1-containing vesicles to the plasmalemma via Munc18–1/syntaxin1/SNAP-25 mediated fusion. *Scientific reports* 2016; 6: 21226. DOI: 10.1038/srep21226.
39. Huang D, Li S, Dhaka A, et al. Expression of the transient receptor potential channels TRPV1, TRPA1 and TRPM8 in mouse trigeminal primary afferent neurons innervating the dura. *Mol Pain* 2012; 8: 66. 20120912. DOI: 10.1186/1744-8069-8-66.
40. Kunkler PE, Ballard CJ, Oxford GS, et al. TRPA1 receptors mediate environmental irritant-induced meningeal vasodilatation. *Pain* 2011; 152: 38-44. 20101113. DOI: 10.1016/j.pain.2010.08.021.
41. Kunkler PE, Zhang L, Johnson PL, et al. Induction of chronic migraine phenotypes in a rat model after environmental irritant exposure. *Pain* 2018; 159: 540-549. DOI: 10.1097/j.pain.0000000000001124.
42. Kunkler PE, Zhang L, Pellman JJ, et al. Sensitization of the trigeminovascular system following environmental irritant exposure. *Cephalalgia : an international journal of headache* 2015; 35: 1192-1201. 20150227. DOI: 10.1177/0333102415574845.
43. Nassar MA, Stirling LC, Forlani G, et al. Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. *Proceedings of the National Academy of Sciences of the United States of America* 2004; 101: 12706-12711. 20040816. DOI: 10.1073/pnas.0404915101.
44. Nielsen CS, Stubhaug A, Price DD, et al. Individual differences in pain sensitivity: genetic and environmental contributions. *Pain* 2008; 136: 21-29. 20070809. DOI: 10.1016/j.pain.2007.06.008.

45. Norbury TA, MacGregor AJ, Urwin J, et al. Heritability of responses to painful stimuli in women: a classical twin study. *Brain* 2007; 130: 3041-3049. 20071011. DOI: 10.1093/brain/awm233.
46. Priest BT, Murphy BA, Lindia JA, et al. Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. *Proceedings of the National Academy of Sciences of the United States of America* 2005; 102: 9382-9387. 20050617. DOI: 10.1073/pnas.0501549102.
47. Zubieta JK, Heitzeg MM, Smith YR, et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science (New York, NY)* 2003; 299: 1240-1243. DOI: 10.1126/science.1078546.
48. Bai G, Ross H, Zhang Y, et al. The Role of DNA Methylation in Transcriptional Regulation of Pro-Nociceptive Genes in Rat Trigeminal Ganglia. *Epigenet Insights* 2020; 13: 2516865720938677. 20200910. DOI: 10.1177/2516865720938677.
49. Crow M, Denk F and McMahon SB. Genes and epigenetic processes as prospective pain targets. *Genome Med* 2013; 5: 12. 20130215. DOI: 10.1186/gm416.
50. Goodin BR, Overstreet DS, Penn TM, et al. Epigenome-wide DNA methylation profiling of conditioned pain modulation in individuals with non-specific chronic low back pain. *Clinical epigenetics* 2022; 14: 45. 20220326. DOI: 10.1186/s13148-022-01265-z.
51. Gombert S, Rhein M, Eberhardt M, et al. Epigenetic divergence in the TRPA1 promoter correlates with pressure pain thresholds in healthy individuals. *Pain* 2017; 158: 698-704. DOI: 10.1097/j.pain.0000000000000815.
52. Takenaka S, Sukenaga N, Ohmuraya M, et al. Association between neuropathic pain characteristics and DNA methylation of transient receptor potential ankyrin 1 in human peripheral blood. *Medicine* 2020; 99: e19325. DOI: 10.1097/md.0000000000019325.
53. Najjar SA and Albers KM. Pain in Inflammatory Bowel Disease: Optogenetic Strategies for Study of Neural-Epithelial Signaling. *Crohns Colitis 360* 2021; 3: otab040. 20210707. DOI: 10.1093/crocol/otab040.
54. Holzer P. Implications of tachykinins and calcitonin gene-related peptide in inflammatory bowel disease. *Digestion* 1998; 59: 269-283. DOI: 10.1159/000007504.
55. Liu Y, Xu F, Wheaton AG, et al. The association between inflammatory bowel disease and migraine or severe headache among US adults: Findings from the National Health Interview Survey, 2015-2016. *Headache: The Journal of Head and Face Pain* 2021; 61: 612-619. https://doi.org/10.1111/head.14087. DOI: https://doi.org/10.1111/head.14087.
56. Engel MA, Leffler A, Niedermirtl F, et al. TRPA1 and substance P mediate colitis in mice. *Gastroenterology* 2011; 141: 1346-1358. 20110718. DOI: 10.1053/j.gastro.2011.07.002.
57. Kun J, Szitter I, Kemény A, et al. Upregulation of the transient receptor potential ankyrin 1 ion channel in the inflamed human and mouse colon and its protective roles. *PloS one* 2014; 9: e108164. 20140929. DOI: 10.1371/journal.pone.0108164.
58. Gombert S, Rhein M, Winterpacht A, et al. Transient receptor potential ankyrin 1 promoter methylation and peripheral pain sensitivity in Crohn's disease. *Clinical epigenetics* 2019; 12: 1. 20191231. DOI: 10.1186/s13148-019-0796-9.
59. Wang Q, Chen K, Zhang F, et al. TRPA1 regulates macrophages phenotype plasticity and atherosclerosis progression. *Atherosclerosis* 2020; 301: 44-53. 20200413. DOI: 10.1016/j.atherosclerosis.2020.04.004.
60. Schreiber AK, Nones CF, Reis RC, et al. Diabetic neuropathic pain: Physiopathology and treatment. *World J Diabetes* 2015; 6: 432-444. DOI: 10.4239/wjd.v6.i3.432.
61. Fagherazzi G, El Fatouhi D, Fournier A, et al. Associations Between Migraine and Type 2 Diabetes in Women: Findings From the E3N Cohort Study. *JAMA Neurol* 2019; 76: 257-263. DOI: 10.1001/jamaneurol.2018.3960.

62. Kong X, Tu Y, Li B, et al. Roux-en-Y gastric bypass enhances insulin secretion in type 2 diabetes via FXR-mediated TRPA1 expression. *Mol Metab* 2019; 29: 1-11. 20190815. DOI: 10.1016/j.molmet.2019.08.009.
63. Killilea T. Long-term consequences of type 2 diabetes mellitus: economic impact on society and managed care. *Am J Manag Care* 2002; 8: S441-449.
64. Wang Z, Ye D, Ye J, et al. The TRPA1 Channel in the Cardiovascular System: Promising Features and Challenges. *Frontiers in pharmacology* 2019; 10: 1253. 20191018. DOI: 10.3389/fphar.2019.01253.
65. He AT, Liu J, Li F, et al. Targeting circular RNAs as a therapeutic approach: current strategies and challenges. *Signal Transduction and Targeted Therapy* 2021; 6: 185. DOI: 10.1038/s41392-021-00569-5.
66. Qureshi RA, Tian Y, McDonald MK, et al. Circulating microRNA Signatures in Rodent Models of Pain. *Mol Neurobiol* 2016; 53: 3416-3427. 20150618. DOI: 10.1007/s12035-015-9281-4.
67. Richner M, Bjerrum OJ, Nykjaer A, et al. The spared nerve injury (SNI) model of induced mechanical allodynia in mice. *J Vis Exp* 2011 20110818. DOI: 10.3791/3092.
68. Lu S, Ma S, Wang Y, et al. Mus musculus-microRNA-449a ameliorates neuropathic pain by decreasing the level of KCNMA1 and TRPA1, and increasing the level of TPTE. *Molecular medicine reports* 2017; 16: 353-360. 20170510. DOI: 10.3892/mmr.2017.6559.
69. Han Q, Liu D, Convertino M, et al. miRNA-711 Binds and Activates TRPA1 Extracellularly to Evoke Acute and Chronic Pruritus. *Neuron* 2018; 99: 449-463.e446. 20180719. DOI: 10.1016/j.neuron.2018.06.039.
70. Berdouk S and Khan S. Migraine with extensive skin markings: a case report. *Int J Emerg Med* 2018; 11: 32. 20180607. DOI: 10.1186/s12245-018-0191-x.
71. Nandyala A and Dougherty C. Dermatologic Symptoms and Syndromes Associated with Headache. *Curr Pain Headache Rep* 2022 20220906. DOI: 10.1007/s11916-022-01080-4.
72. Oaklander AL. Neuropathic itch. *Semin Cutan Med Surg* 2011; 30: 87-92. DOI: 10.1016/j.sder.2011.04.006.
73. Park CK, Xu ZZ, Berta T, et al. Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. *Neuron* 2014; 82: 47-54. DOI: 10.1016/j.neuron.2014.02.011.
74. Lehmann SM, Rosenberger K, Krüger C, et al. Extracellularly delivered single-stranded viral RNA causes neurodegeneration dependent on TLR7. *Journal of immunology (Baltimore, Md : 1950)* 2012; 189: 1448-1458. 20120627. DOI: 10.4049/jimmunol.1201078.
75. Winkler CW, Taylor KG and Peterson KE. Location is everything: let-7b microRNA and TLR7 signaling results in a painful TRP. *Science signaling* 2014; 7: pe14. 20140527. DOI: 10.1126/scisignal.2005407.
76. Zhao J, Lee MC, Momin A, et al. Small RNAs control sodium channel expression, nociceptor excitability, and pain thresholds. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2010; 30: 10860-10871. DOI: 10.1523/jneurosci.1980-10.2010.
77. Liu X, Yang W, Zhu C, et al. Toll-like receptors and their role in neuropathic pain and migraine. *Mol Brain* 2022; 15: 73. 20220820. DOI: 10.1186/s13041-022-00960-5.
78. Biondi DM. Is migraine a neuropathic pain syndrome? *Curr Pain Headache Rep* 2006; 10: 167-178. DOI: 10.1007/s11916-006-0042-y.
79. Chakravarty A and Sen A. Migraine, neuropathic pain and nociceptive pain: towards a unifying concept. *Medical hypotheses* 2010; 74: 225-231. 20090917. DOI: 10.1016/j.mehy.2009.08.034.
80. Vuralli D, Wattiez A-S, Russo AF, et al. Behavioral and cognitive animal models in headache research. *The journal of headache and pain* 2019; 20: 11. DOI: 10.1186/s10194-019-0963-6.