Brain barriers and their potential role in migraine pathophysiology

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Abstract
Migraine is a ubiquitous neurologic disease that afflicts people of all ages. Its molecular pathogenesis involves peptides that promote intracranial vasodilation and modulate nociceptive transmission upon release from sensory afferents of cells in the trigeminal ganglion and parasympathetic efferents of cells in the sphenopalatine ganglion. Experimental data have confirmed that intravenous infusion of these vasoactive peptides induce migraine attacks in people with migraine, but it remains a point of scientific contention whether their site of action lies outside or within the central nervous system. In this context, it has been hypothesized that transient dysfunction of brain barriers before or during migraine attacks might facilitate the passage of migraine-inducing peptides into the central nervous system. Here, we review evidence suggestive of brain barrier dysfunction in migraine pathogenesis and conclude with lessons learned in order to provide directions for future research efforts.

Keywords: Headache, Trigeminovascular system, Blood-brain barrier, Aura

Introduction
Migraine is a prevalent neurological disorder that is characterized by recurrent headache attacks of moderate to severe intensity and accompanying symptoms such as nausea, vomiting, photo-, and phonophobia [1]. Its pathogenesis is to be explained within the framework of the trigeminovascular system [2]. This system includes the trigeminal ganglion and its peripheral axonal projections that innervate pain-sensitive intracranial structures, e.g. meninges [3]. In addition, central axonal projections arise from trigeminal ganglion cells and convey nociceptive impulses to second-order trigeminovascular neurons in the brain stem [3]. These neurons, in turn, project to third order trigeminovascular neurons in the thalamus, which then convey nociceptive impulses to a wide array of cortical areas that are involved in pain processing, e.g. the somatosensory cortex [3].

A point of scientific contention is whether the molecular mechanisms that initiate migraine attacks lie outside or within the central nervous system (CNS) [1]. Upon activation, peripheral projections of the trigeminal nerve release neurotransmitters that elicit vasodilation and modulate nociceptive transmission, e.g. calcitonin gene-related peptide (CGRP) and pituitary adenylate cyclase-activating polypeptide (PACAP) [3]. Intravenous administration of these neurotransmitters can induce migraine attacks in individuals with migraine, whereas healthy volunteers most often develop no more than mild headache [4]. Based on this, it becomes a question of key interest whether these neuropeptides can cross the blood-brain barrier (BBB) and initiate migraine attacks from within the CNS. If not, this would favor a peripheral origin of migraine.

In this Review, we examine evidence suggestive of brain barrier dysfunction in migraine. Furthermore, we discuss whether neuropeptides that induce migraine...
attacks have their site of action within the CNS. Lastly, we review some of the outstanding research questions and provide directions for future research efforts.

**Brain barriers**
The brain has multiple barriers to restrict non-selective passage of solutes into brain parenchyma [5]. In the meninges, the arachnoid barrier impedes the leakage of solutes from fenestrated blood vessels into the subarachnoid space that is filled with cerebrospinal fluid (CSF). Blood vessels in the subarachnoid space consist of endothelial cells that are connected by tight junctions with similar barrier characteristics as blood vessels in brain parenchyma but without surrounding pericytes and astrocytic end-feet [6]. This hinders passage of solutes from the blood to the CSF and is called the blood-CSF-barrier (BCSFB). The arterioles that branch from the subarachnoid blood vessels penetrate the brain parenchyma and constitute the brain microvasculature. The microvasculature is part of the BBB, a dynamic interface comprised of vascular cells (e.g. endothelium, pericytes), glial cells (e.g. astrocytes), and neurons [5–7].

As a rule of thumb, hydrophilic molecules of less than 620 Da cross the BBB via diffusion along the paracellular route, and small lipophilic molecules diffuse freely through the lipid membranes. However, the majority of these freely diffusing lipophilic molecules are rapidly removed from endothelial cells by efflux transporters and do not reach the brain parenchyma. All other solutes require transporters located on endothelial cells [5]. Solutes also enter the CNS via the circumventricular organs (CVOs) that are free of BBB and located near the ventricular system [8]. The CVOs include the following structures: area postrema, median eminence, pineal gland, pituitary gland, subcommissural organ, subfornical organ, and vascular organ of lamina terminalis [8]. Their leakiness allows accumulation of circulating agents, but a barrier comprised of tanyocytes with tight junctions prevents the passage of agents into the CSF [6].

**Blood-brain barrier dysfunction in migraine pathogenesis**
The hypothesis of BBB dysfunction in migraine was first proposed by Harper and colleagues in 1977 [9]. The authors speculated that a leaky BBB allowed circulating agents in the peripheral blood to enter the CNS and facilitate transmission of nociceptive impulses that ultimately yield the perception of migraine pain. However, there is currently very limited experimental evidence in favor of this hypothesis. Three magnetic resonance imaging (MRI) studies found no evidence of a leaky BBB during and outside of spontaneous migraine attacks (Table 1) [10–12]. Two of the studies used gadolinium-based dynamic contrast-enhanced (DCE) MRI to assess disruption of the BBB in five regions of interest, being the anterior, middle, and posterior cerebral area, brain stem, posterior pons, and whole brain [10, 11]. Patients were scanned during a spontaneous migraine attack as well as on an attack-free day. No changes suggestive of BBB dysfunction were identified in 19 patients with migraine without aura [14] or 19 patients with migraine with aura [15] when comparing data during and outside of migraine attacks. There was also no association between BBB permeability and any headache feature (e.g., location, intensity). However, post hoc power analysis showed that BBB permeability changes of less than 35% in patients with migraine without aura and changes of less than 11% in patients with migraine with aura could not be excluded [10, 11]. Another limitation is that early and/or transient changes in BBB permeability may not have been detected, as median time from onset of attack to MRI scan was 6.5 h in patients with migraine without aura [14] and 7.6 h in patients with migraine with aura [15]. In a third DCE-MRI study, differences in BBB permeability were assessed in 35 patients with migraine with/without aura and 21 healthy non-headache controls [12]. Patients with migraine were scanned outside of attacks and the authors found no changes in BBB permeability when comparing the two groups. Although they did find a lower fractional plasma volume in the left amygdala of patients with migraine when compared with healthy controls [12], it is unclear whether this finding has any relevance to BBB dysfunction during migraine attacks.

BBB permeability has also been assessed during provoked migraine attacks using positron emission tomography – computed tomography (PET-CT) with the radioligand $^{11}$C-dihydroergotamine ($^{11}$C-DHE) (Table 1) [13]. Migraine attacks were induced by intravenous infusion of the nitric oxide donor glyceryl trinitrate (GTN) which is a potent vasodilator known to provoke migraine attacks in 80% of patients with migraine [16]. It should be noted that patients were eligible for study inclusion only if they developed a migraine attack after GTN infusion whereas subjects in the control group had to remain free of pain following GTN infusion [13]. The authors reported no changes suggestive of BBB dysfunction when comparing scans before and during provoked attacks, or when comparing scans of patients to those of controls. However, the limited spatial resolution of PET and the usage of $^{11}$C-DHE tracer (584 Da) might impede the detection of minor changes in BBB permeability [13]. Taken together, it seems evident that neuroimaging studies provide no evidence for BBB dysfunction during migraine attacks, although early transient or minor changes in BBB permeability cannot be fully excluded.

Disfunction of the BBB has been evaluated by the activity of matrix metalloproteinases (MMPs) since some
members of this protease family seem to be implicated in breakdown of the BBB [17]. In a rodent study, cortical spreading depression (CSD) led to BBB disruption and an increase in MMP-9 levels in cortical homogenates ipsilateral to the induced CSD [18]. However, CSD was induced by three pinpricks after removing large parts of the calvarium bilaterally and opening the dura mater. This procedure had evidently caused neuroinflammatory responses which, in turn, appears to increase the firing rate of neurons in the meninges overlying the occipital lobe during migraine with visual aura in 11 migraine patients [28]. Repetitive episodes of neuroinflammation in migraine patients could result in a leaky BBB and allow passage of inflammatory mediators to the brain parenchyma.

### Table 1: Human experimental studies of BBB integrity in migraine

| Study | Method | Study population | Outcomes | Limitations |
|-------|--------|------------------|----------|-------------|
| Amin et al., 2017 [10] | Gadolinium-based-DCE-MRI at rest and during spontaneous migraine attacks. Permeability assessed in five different brain regions located in the anterior, middle, and posterior cerebral area, brain stem and posterior pons. | 19 MO | No changes in BBB permeability on attack versus headache-free days. No changes in BBB permeability between pain and non-pain side. | Power of study caused a detection limit of 35%. Permeability assessed using a 604 Da hydrophilic molecule. Median time of onset of attack to scan was 6.5 h. |
| Hougaard et al. 2017 [11] | Gadolinium-based-DCE-MRI at rest and during spontaneous migraine attacks. Permeability assessed in five different brain regions located in the anterior, middle, and posterior cerebral area, brain stem and posterior pons. | 19 MA | No changes in BBB permeability on attack versus headache-free days. No changes in BBB permeability between pain and non-pain side. No difference in affected or non-affected hemispheres. | Power of study caused a detection limit of 1%. Permeability assessed using a 604 Da hydrophilic molecule. Median time of onset of attack to scan was 7.6 h and no patients were scanned during aura symptoms. |
| Kim et al., 2019 [12] | Gadolinium-based-DCE-MRI performed on migraine patients outside of attacks and compared with scans of healthy controls | 21 MA, 14 MO, 21 Healthy controls | No difference in gadolinium BBB permeability between patients and controls. Lower fractional plasma volume in left amygdala in migraine patients | Permeability assessed using a 604 Da hydrophilic molecule. Age of control group was not matched with migraine group. Changes in amygdala cannot be directly correlated to changes in BBB integrity. |
| Schankin et al., 2016 [13] | PET-scan and the radioligand 11C-DHE at rest and during GTN-induced migraine attacks. | 2 MA, 4 MO, 6 Healthy controls | No binding of the radioligand to brain parenchyma at rest or during GTN-induced attacks in migraineurs or healthy controls. | Limited spatial resolution of PET. Permeability assessed with 11C-DHE with a molecular size of 583.7 g/mol. GTN-induced migraine attacks instead of spontaneous attacks. |

BBB Blood-Brain Barrier, Da Dalton, DCE-MRI Dynamic Contrast-Enhanced Magnetic Resonance Imaging, GTN Glycerol trinitrate, H Hour, MO Migraine without aura, MA Migraine with aura, PET Positron Emission Tomography
neuropeptides into the brain parenchyma [29, 30]. Further studies are needed to evaluate whether CSD-induced inflammatory processes are associated with changes in brain barrier permeability.

**Provoked migraine attacks**

The trigeminovascular system is widely considered the anatomical and physiological substrate of migraine pathogenesis [1]. Within this framework, parasympathetic efferents of cells in the sphenopalatine ganglion and sensory afferents of cells in the trigeminal ganglion release, upon activation, various peptides that promote dilation of intracranial arteries and modulate nociceptive transmission [1]. Decades of research have established that intravenous infusion of certain naturally occurring peptides can induce migraine attacks in patients with migraine while healthy volunteers develop most often no more than a mild headache [4]. This raises the question of whether these peptides induce migraine attacks outside or within the CNS.

The following peptides have been implicated in migraine pathogenesis [31]: adrenomedullin (ADM), amylin, calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide (PACAP), and vasoactive intestinal polypeptide (VIP). All are potent vasodilators and induce migraine attacks when administered by intravenous infusion to patients with migraine [31, 32]. They mediate their effects via G protein-coupled receptors that, in turn, activate the cyclic adenosine monophosphate (cAMP)-dependent signaling pathway [31]. Preclinical evidence suggests that this pathway results in the opening of ATP-sensitive potassium (K\textsubscript{ATP}) channels, and it has been hypothesized that opening of potassium channels might be the final common pathway in the genesis of a migraine pain [1]. Collectively, the neuropeptides have receptor-binding sites that are expressed at multiple levels of the trigeminovascular system (Table 2) of which the extracerebral vasculature, extracranial vasculature and the trigeminal ganglion is not brain barrier protected.

Direct binding of neuropeptides to A\textsubscript{δ}-fibers or neurons in the trigeminal ganglion and subsequent hyperexcitability has been suggested as the pain-initiating mechanism in migraine. However, based on the suggested intracellular pathway with K\textsubscript{ATP} channels as the end station direct binding to nerve fibers would result in hyperpolarization, and thus the vasculature might be a more relevant site of action. Other ganglia without barrier protection may also be involved in migraine pathogenesis, and preclinical data has suggested that activation of the sphenopalatine ganglion causes release of PACAP and VIP from its efferent fibers [1]. This mechanism is bypassed in provoked migraine attacks where the neuropeptides are given intravenously but could play a role in spontaneous attacks. It merits emphasis that ADM, amylin, and CGRP belong to the same family of peptides [33]. The same is also true for PACAP and VIP [34].

The molecular size of the aforementioned peptides suggests a very limited ability to cross the brain barrier (Table 3) [5]. This accords with animal studies that have radiolabeled all peptides, except for CGRP, and quantified the degree of BBB passage [40, 41, 43, 50, 69–71]. In rodents, injection of radiolabeled ADM does not cross the BBB [69], whilst the peak brain uptake of injected radiolabeled amylin is 0.11–0.13% [40, 70]. Furthermore, injection of radiolabeled PACAP yields a brain uptake of less than 0.07% in rodents for both its isoforms (i.e. PACAP-38 and PACAP-27) [43], while injection of radiolabeled VIP results in brain uptake of 0.15% in mice and no brain uptake in rats [41, 50].

Another method to investigate possible brain barrier passage of migraine-inducing peptides involves the study of their vascular responses. In this context, dilation of the middle cerebral artery (MCA) has often been used as a surrogate marker of BBB penetration. The MCA is surrounded by the BCSFB and branches into smaller vessels that penetrate the brain parenchyma. These small vessels are surrounded by the BBB and constitute the cerebral microvasculature. Based on this, it is assumed that lack of MCA dilation by migraine-inducing peptides would suggest no or very limited BBB passage. This, in turn, would indicate that the site of action of migraine-inducing peptides is located outside of the CNS.

The vascular responses of migraine-inducing peptides have been extensively studied in vitro using rodent or human MCA. These studies have found that ADM, CGRP, both PACAP isoform, and VIP did not elicit dilation of the rat MCA following luminal application [35, 45, 48, 59], whilst amylin induced only a weak dilator response [71]. Although these preclinical studies provide evidence against a central side of action of migraine-inducing substances, it should be recognized that tissue preparation can affect transporters located in the endothelium which, in turn, might affect tissue permeability.

This issue is avoided when assessing vasodilation in vivo by using ultrasound or magnetic resonance angiography (MRA). Ultrasound of brain arteries detects changes in blood velocity, a factor inversely proportional to the diameter of the blood vessel. Since the blood flow is also dependent on the vascular diameter, a decrease in MCA velocity only reflects vasodilation if the single photon-emission computed tomography (SPECT)-determined regional cerebral blood flow (rCBF) is unchanged [54].
A decrease in ultrasound assessed MCA velocity and no change in SPECT-determined rCBF has been reported after CGRP and VIP infusion [36, 51] whereas infusions of ADM did not affect any of the two parameters [39]. This suggests CGRP- and VIP-induced vasodilation of the MCA and possibly BCSFB passage. A decrease in MCA velocity was also reported after infusion of PACAP-38, and PACAP-38 has previously been reported not to affect rCBF in healthy volunteers [47, 72]. Although some of the ultrasound-based studies indicate BCSFB permeability, the results should be interpreted with caution since this method assesses vasodilation indirectly.

MRA enables visualization of extra- and intracerebral arteries and direct measurement of arterial circumferences. MRA studies performed on healthy controls and migraine patients reported dilation of the MMA following administration of telcagepant at an efficacious dose – suggestive of a peripheral site of action for telcagepant [76]. This finding accords well with the observation of reduced mechanical sensitivity thresholds in rodents following intraperitoneal, but not intracerebroventricular, injection of olcegepant and a mAb against CGRP [77]. Furthermore, intravenous injection of fluorescently-labeled fremanezumab yielded labeling of sensory and autonomic ganglia as well as the dura mater, whereas no fluorescent signal was observed in structures within the CNS [78].

Collectively, it seems evident that therapies targeting CGRP-signaling are unlikely to cross the BBB which, in turn, indicates that BBB passage is not needed to achieve therapeutic benefits with medications for migraine. It might indeed be advisable to develop drugs that do not cross the BBB to avoid adverse effects.

### Table 2

Receptor binding sites within the trigeminovascular system. The table gives an overview of seven different migraine-inducing substances and their various binding sites within the trigeminovascular system. In this table, the trigeminovascular system is divided into the following structures: extracranial vasculature, intracranial vasculature, the trigeminal ganglion, the spinal trigeminal nucleus, and thalamus. The binding sites have been detected by usage of polymerase chain reaction, in-situ hybridization, western blot, or immunostaining in human, monkey, pig, or rodent tissues.

| Studied substance | Extracranial vasculature | Intracranial vasculature | Trigeminal ganglion | Spinal trigeminal nucleus | Thalamus |
|------------------|--------------------------|--------------------------|---------------------|--------------------------|----------|
|                  | Extracerebral | Intracerebral | Extracerebral | Intracerebral | Extracerebral | Intracerebral | Extracerebral | Intracerebral | Extracerebral | Intracerebral |
| CGRP [33, 88-92] | H | R | H | R | H | R | H | R | H | H |
| Adrenomedullin [33, 88, 91, 93] | H | R | H | R | H | R | H | R | H | H |
| Amylin [33, 91] | H | R | H | R | H | R | H | R | H | R |
| PACAP [34, 94-97] | R | H | R | H | R | H | R | H | R | M |
| VIP [34, 95, 97] | R | H | R | H | R | H | R | H | R | R |
| Levocromakalim [98-102] | R | H | P | R | R | R |
| MaxiPost [103-105] | R | R | R | R | R | R | R | R | R | R |
associated with CNS depression. For example, lasmiditan (serotonin (5-HT) 1F receptor agonist) is an acute medication for migraine that can cause CNS-related side effects (incl. Dizziness, sedation, and temporary driving impairment) which are likely to limit its use in clinical practice [79–81].

### Outstanding research questions

The current evidence obtained from both neuroimaging and biochemical markers in humans suggests no disruption of the brain barriers in migraine. However, the limited sensitivity of the applied methods requires more studies to assess the relationship between brain barrier dysfunction and migraine pathophysiology. Future studies could use the newly developed sensitive modified DCE-MRI method that considers the arterial input function and cerebral blood flow [82] since both these parameters could be affected in migraine. This method has identified BBB dysfunction in early stages of cognitive dysfunction [82]. Additionally, soluble PDGFRβ, a biomarker of BBB pericyte injury, could be analyzed in migraine patients [83].

The limited brain barrier passage of migraine-inducing neuropeptides suggests a peripheral origin of migraine. However, migraine attacks can also be induced in migraine patients by administration of vasoactive molecules with BBB permeability (e.g. GTN or cilostazol [16, 61, 62]), and several questions concerning migraine origin remain unanswered. One of them is the presence of premonitory symptoms (PS) in migraine which might be suggestive of initial activation of central structures in migraine attacks. The underlying mechanisms of PS are still unclear. Infusion of GTN to migraine patients induced PS in 36% (12/33) of patients prior to triggered migraine attacks [84]. In another study, GTN was found to induce PS in a selected group of patients known to have migraine with PS while PET-scans showed activation in various different brain areas, including hypothalamus [85]. In this study, however, no control group was included, and thus changes may relate to GTN administration rather than migraine. Furthermore, none of these studies compared PS in patients who reported and did not report migraine attacks. A study assessing the incidence of PS in migraine patients after administration of

### Table 3 Brain Barrier Permeability of Migraine-Inducing Substances

| Substance     | Size (Dalton) | Permeability | Migraine induction rate | MCA changes in rodents | MCA changes in humans |
|---------------|---------------|--------------|-------------------------|------------------------|------------------------|
|               |               |              |                         | In vitro               | In vivo                | In vivo                |
|               |               |              |                         | Changes after luminal administration in vascular models | Assessed by ultrasound and SPECT | Assessed by MRA |
| CGRP          | 3791.3        | Unknown      | 57% [31]                | No dilation [35]       | MCA velocity drop [36] | rCBF no changes [36]  |
| Adrenomedullin| 6028.8        | Unknown      | 55% [38]                | No dilation [35]       | MCA no changes [39]    | rCBF no changes [39]  |
| Amylin        | 3904.5        | 0.11–0.13% Inj/g brain (rodents) [40, 70] | 41% [42]                | Weak dilatory response [35] | Unknown               | Unknown               |
| PACAP27       | 3147.6        | 0.066% in brain parenchyma (rodents) [43] | 55% [44]                | No dilation [45]       | Unknown               | No changes [46]        |
| PACAP38       | 4534.3        | 0.053% in brain parenchyma (rodents) [43] | 58% [47]                | No dilation [45, 59]   | MCA velocity drop [47] | rCBF not measured [47] |
| VIP           | 3326.8        | None (rodents) [50] | 0.15% Inj/g brain (rodents) [41] | 71% [32]                | No dilation [45, 59]   | MCA velocity drop [51, 52] | rCBF no changes [51]  |
| GTN           | 227.09        | Yes          | 80% [16]                | Unknown                | MCA velocity drop [54] | rCBF no changes [54]  | Dilation [55, 56]     |
| Sildenafil     | 474.6         | 0.028% Inj/g brain (rodents) [57] | 83% [58]                | No dilation [59, 60]   | MCA no changes [58]    | rCBF no changes [58]  | Unknown               |
| Cilostazol    | 369.5         | Yes          | 86% [61], 88% [62]      | Unknown                | MCA velocity drop [63] | rCBF no changes [63]  | Dilation [64]         |
| Levocromakalim| 286.3         | Yes          | 100% [65]               | Unknown                | MCA velocity drop [68] | rCBF not measured [68] | Unknown               |
| MaxiPost      | 359.7         | Yes          | 95% [67]                | Unknown                | MCA velocity drop [68] | rCBF not measured [68] | Unknown               |

CGRP: Calcitonin Gene-Related Peptide, GTN: Glyceryl Trinitrate, Inj/g: Injection/g, MCA: Middle Cerebral Artery, MRA: Magnetic Resonance Angiography, PACAP: Pituitary adenylate cyclase-activating peptide, rCBF: Regional Cerebral Blood Flow, SPECT: Single Photon Emission Computed Tomography, VIP: Vasoactive Intestinal Peptide. Molecular sizes obtained from PubChem (pubchem.ncbi.nlm.nih.gov)
trigeminal signaling molecules reported no PS after CGRP infusion but PS in 48% of patients after PACAP38 infusion [86]. However, CGRP and PACAP38 did not induce more PS in patients who developed an attack compared to those who did not develop an attack [86], and this aspect must be studied in healthy subjects. Further studies are needed to clarify the presence of a premonitory phase in migraine which may contribute to the discussion of migraine origin.

Additionally, several outstanding questions relate to migraine aura. Although CSD is accepted as the substrate of migraine aura, it is still unknown how CSD arises in a seemingly otherwise healthy cerebral cortex of migraine patients, and how it is related to the headache phase of migraine. The unpredictable and short-lasting nature of migraine aura makes it difficult to study patients during symptoms and thereby answer outstanding research questions on this matter. However, recently a randomized, double-blind, placebo-controlled, cross-over study reported that administration of the K_{ATP}-channel opener levcromakalim induced aura in 10 of 17 (59%) patients suffering from migraine with aura and migraine attacks in 14 of 17 (82%) the patients [87]. The authors suggest that K_{ATP}-channel opening most likely induces CSD and migraine headache via separate pathways since levcromakalim efficiently triggers migraine without aura [65] and this even in some patients who have previously experienced aura symptoms during all their migraine attacks [87]. However, the trigger of migraine aura is still unknown and future research efforts are required to fully understand the initiation CSD and its relation to the headache phase of migraine.

**Conclusion**

Brain barrier disruption has been hypothesized to play an important role in the genesis of migraine attacks. The current evidence suggests, however, that there is limited experimental data in favor of this hypothesis. Nonetheless, it cannot be excluded that, in particular, CSD might be associated with inflammatory processes within the brain and meninges, ultimately causing transient brain barrier disruption. Further studies are warranted to ascertain whether early transient changes in BBB permeability occur during the early phases of a migraine attack.

**Abbreviations**

CNS: Central nervous system; CGRP: Calcitonin gene-related peptide; PACAP: Pituitary adenylate cyclase-activating peptide; BBB: Blood-brain barrier; CSF: Cerebrospinal fluid; BCSFB: Blood-cerebrospinal fluid barrier; CVO: Circumventricular organs; MRI: Magnetic resonance imaging; DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging; PET-CT: Position emission tomography-computed tomography; GTN: Glyceryl trinitrate; 11C-DHE: 11C-Dihydroergotamine; MMP: Matrix metallopeptidase; CSD: Cortical spreading depression; ADW: Adrenomedullin; VIP: Vasoactive intestinal peptide; cAMP: Cyclic adenosine monophosphate; K_{ATP}: ATP-sensitive potassium channel; MCA: Middle cerebral artery; MRA: Magnetic resonance angiography; SPECT: Single-photon emission computerized tomography; rCBF: Regional cerebral blood flow; BOLD: Blood-oxygen-level-dependent; PS: Premonitory symptoms; mAb: monoclonal antibody

**Authors’ contributions**

Drafting of the manuscript and graphic items: A.W., H.A. and M.A. Critical revision of intellectual content: N.H., A.S., B.Z. and M.L. All authors read and approved the final manuscript.

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**Competing interests**

A.W., H.A., N.H., A.S. B. Z. and M. L. all declare that they have no competing interests. M.A. is a consultant, speaker, or scientific advisor for AbbVie, Allergan, Amgen, Eli Lilly, Lundbeck, Novartis, and Teva and a primary investigator for ongoing AbbVie/Allergan, Amgen and Lundbeck trials. M.A. has no ownership interest and does not own stocks of any pharmaceutical company. M.A. serves as associate editor of Cephalalgia, associate editor of the Journal of Headache and Pain, and associate editor of Brain.

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