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REVIEW



Targeting pituitary adenylate cyclase-activating polypeptide (PACAP) with monoclonal antibodies in migraine prevention: a brief review

Cecilia Rustichelli ^a, Flavia Lo Castro ^b, Carlo Baraldi ^c and Anna Ferrari ^d

^aDepartment of Life Science, University of Modena and Reggio Emilia, Modena, Italy; ^bSchool of Pharmacology and Clinical Toxicology, University of Modena and Reggio Emilia, Modena, Italy; ^cDepartment of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy; ^dUnit of Medical Toxicology, Headache Centre and Drug Abuse; Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

ABSTRACT

Introduction: Interest is growing in the role of pituitary adenylate cyclase-activating polypeptide (PACAP) and its specific PAC1 receptor in migraine and in their antagonism as a strategy for migraine prevention.

Areas covered: We discuss and critically evaluate (i) the evidence of the role of PACAP in migraine pathophysiology and (ii) the first clinical trials in migraine prophylaxis with monoclonal antibodies AMG 301 and ALD1910 which act against PAC1 and PACAP38 respectively. We examined PubMed, Scopus, and ClinicalTrials.gov electronic databases to examine the relevant material.

Expert opinion: There is much proof of the ability of PACAP to cause migraine, but there is limited evidence that blocking PACAP or PAC1 receptor can prevent migraine. However, the potential of anti-PACAP antibodies in migraine prophylaxis is high. Theoretically, if these antibodies block the activation of the trigeminovascular system, they will prevent the onset of migraine attacks. There are still knowledge gaps in the role of PACAP in migraine and the risk/benefit ratio of anti-PACAP antibodies must be carefully studied.

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1. Introduction

Among the most painful and disabling disorders there is migraine, which causes a very high load of individual suffering, enormous social costs and a strong need for better pharmacotherapies. In fact, the treatment of migraine was mainly based on nonspecific drugs that are not suitable for all patients because of side effects [1]. Migraine is a primary disorder characterized by recurrent attacks of throbbing headache, associated with nausea, vomiting, photophobia, and phonophobia [2], which affects more than 12% of the general population, with a clear prevalence of the female gender [3].

The etiology of migraine is still not fully known; it is thought to be a neurovascular disorder characterized by an increased cortical excitability, brain stem as generator, the origin is perhaps in the hypothalamus, and activator (unclear how) of the trigeminovascular system [4].

The activation of trigeminal sensory pathways is thought to cause an inflammatory response involving immune, vascular, and neuron cells, with release of neuropeptides and pro-inflammatory neurotransmitters, which induce a state of sterile inflammation in the intracranial meninges, leading to further activation of nociceptors and migraine pain [5–8]. Although neuroinflammation in the trigeminovascular system does not completely explain the onset of the attack, it could be crucial in promoting the progression of episodic migraine into a chronic form [9]. Various sensory neuropeptides, including calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP),

and pituitary adenylate cyclase-activating polypeptide (PACAP), play an important role in the pathophysiology of migraine [10].

Better understanding these various systems involved in migraine pioneered the development and marketing of innovative treatments targeting CGRP [11]. In turn, these recent successes have further stimulated the search for novel migraine therapies [12]. In particular, ongoing research is focusing on the involvement of PACAP in migraine pain. The purpose of this review is to present an update on current developments in targeting PACAP for migraine prophylaxis. Moreover, we critically discussed this area.

A literature search on migraine and PACAP was performed. The electronic databases PubMed, Scopus, and ClinicalTrials.gov were used to identify the background and relevant studies (preclinical and clinical). In particular, database search was conducted by entering the keyword ‘migraine’ in combination with the following terms: clinical trial; PACAP; PAC1; PACAP38; PACAP27; prophylactic treatment; trigeminovascular system; VIP; VPAC1; and VPAC2. Search results were assessed for their overall relevance to this review.

2. PACAP background

PACAP is a neuropeptide that shows very high homology with VIP. It belongs to the glucagon/secretin superfamily of peptides (like VIP and secretin) and shares 68% and 37% homology of its amino acid sequence with VIP and secretin, respectively. Two bioactive forms exist: a 38 amino acid form

Article highlights

- There is much evidence suggesting a key role of PACAP in the PAC1 receptor-mediated activation of the trigeminovascular system which is central in the triggering of the migraine attack
- Clinical trials in migraine prophylaxis have been initiated with the monoclonal antibodies AMG 301 and ALD1910 which act against the PAC1 receptor and PACAP38 respectively
- Anti-PACAP38 or anti-PAC1 antibodies in migraine prevention have potential; theoretically, if these antibodies demonstrate the capacity to block trigeminovascular system activation, they should be capable of preventing the onset of migraine attacks
- To utilize anti-PACAP drugs in migraine prophylaxis, their long-term safety must be examined and clarified. Moreover, their advantages should be compared with those of the anti-CGRP monoclonal antibodies that are available

(PACAP38) and a 27 amino acid form (PACAP27). The amino acid sequence of mature human PACAP38 is identical in all mammals, suggesting that it was highly conserved during evolution [13]. The ADCYAP1 gene, on chromosome 18, encodes for a pre-proprotein called 'prepro-PACAP' that is further metabolized in PACAP38 whose internal cleavage leads to the formation of PACAP27 [14]. PACAP is a pleiotropic peptide that has been found in the respiratory, gastrointestinal, urinary, reproductive, and endocrine systems [15]. It is well represented in the central and peripheral nervous system, where it acts as a neuromodulator, immunomodulator, and neurotransmitter [16]. PACAP38 is the most frequent form in brain tissue [15]; this polypeptide can cross the blood brain barrier (BBB) by active transport, but it is rapidly degraded or refluxed into the blood, so a direct effect on the central nervous system (CNS), in particular on nociception, is more likely due to the activation of an area not protected by BBB [17].

G-protein coupled PACAP receptors are PAC1, VPAC1, and VPAC2. VPAC1 and VPAC2 bind PACAP38, PACAP27, and VIP with approximately equal affinity, while PAC1 has a much higher affinity for PACAP38 and PACAP27 [15,18]. These receptors activate adenylyl cyclase producing a cyclic adenylyl cyclase monophosphate/protein kinase A cascade, which can in turn activate the mitogen-activated protein kinase. The PAC1 receptor is also coupled to phospholipase C, which stimulates calcium mobilization and protein kinase A activation [19]. The PAC1 receptor gene can originate different isoforms by an alternative splicing [20].

Immunohistochemical studies confirmed the presence of PACAP in critical areas for the pathophysiology of migraine, such as the trigeminal system, including the trigeminal ganglion (TG) [21] and the trigeminal nucleus caudalis (TNC) [22]; moreover PACAP was found in nerve fibers of the dura mater and cerebral vessels [23], cervical spinal cord [22], sphenopalatine and otic ganglia [24], and in different hypothalamic and brainstem nuclei [25,26]. In accordance with the presence of the polypeptide, PACAP receptors are distributed in different regions of the CNS [27,28].

2.1. Role of PACAP in migraine

Primarily, the ability of PACAP38 to induce headache was tested in order to study its role in migraine. In a first study [29], ten out of twelve healthy subjects reported headache after PACAP38 administration. Subsequently [30], a double-blind, randomized, and placebo-controlled study found that intravenous administration of PACAP38, but not of placebo, caused headache in healthy subjects and migraineurs without aura and a migraine-like attack in 58% of migraineurs without aura and only in 16% of controls. It was also found a dilatation of the middle cerebral artery (MCA) and the superficial temporal artery (STA), recorded by a transcranial Doppler and ultrasonography, respectively. A magnetic resonance (MR) angiography study infusing PACAP38 in nine healthy volunteers showed that 89% of the participants experienced an immediate headache and 100% experienced a delayed headache. Furthermore, PACAP38 induced a long-lasting dilatation of the extracranial part of the middle meningeal artery (MMA), but no dilatation of the MCA. These effects were sensitive to sumatriptan injection (6 mg) [31]. In a second MR angiography study, PACAP38 infusion induced headache in 73% of migraine patients, while VIP infusion induced headache in only 18% of patients. Furthermore, PACAP38 provoked long-lasting vasodilatation of extracranial arteries (such as MMA and STA), but not of intracranial ones (such as MCA) [32]. To deepen knowledge on PACAP38 activity, a resting-state functional MR study investigated three networks (salience, sensorimotor, and default mode), implicated in cognition, pain processing, photo and phonophobia, and emotional processing, during the early phase of migraine attacks pharmacologically-induced by PACAP38 and VIP. Only intravenous PACAP38 infusion, and not VIP, was associated with changes in brain network connectivity, such as increased connectivity in areas involved in the salience network and decreased/increased connectivity in areas involved in the sensorimotor and default mode network [33]. It was also speculated that PACAP38 was involved in the development of premonitory symptoms of migraine attacks because these symptoms appeared after PACAP38 and CGRP infusions in 48% and 9% of migraine patients, respectively [34]. Other evidence of a connection between migraine and PACAP was the finding of higher levels of PACAP38 in the ictal period compared to the interictal period [35,36] and the decrease of these levels in patients with migraine after sumatriptan administration [36]. In addition, two studies reported lower interictal levels of PACAP38 in migraineurs than in controls, a phenomenon attributed to chronic depletion of PACAP38 caused by an excessive consumption during the attacks [35,37]. A clinical trial (NCT02542605) evaluated the connections between CGRP and PACAP38, blocking CGRP release by its antagonist AMG 334 (erenumab), but no correlation was reported: hence the hypothesis that PACAP38 may represent a unique and distinct pathway of migraine [38]. At last, a randomized, double-blind, placebo-controlled trial (NCT03881644) has been completed to investigate the incidence of headache, migraine attacks, and flushing after PACAP38 infusion, with and without treatment with sumatriptan in migraineurs, but it has not been published yet. Recently, the infusion of PACAP27 in migraine

patients provoked in a crossover study significantly more migraine-like attacks than placebo, further strengthening the role of PACAP in the pathogenesis of migraine [39].

Considering that PAC1 receptors are more selective for PACAP than VPAC1 and VPAC2 receptors, it was hypothesized that PACAP38-induced migraine was mediated by the PAC1 receptor and caused by prolonged vasodilatation of extracranial vessels [18], perhaps due to mast-cell degranulation [40–42]. However, two provocation studies in humans with PACAP38 infusion found no increase in markers for mast cell degranulation (serum tryptase and tumor necrosis factor alpha) [32,43].

PACAP could be involved in the activation/sensitization of the trigeminovascular system, too. Indeed, the TG and the TNC contain PACAP38 [21,22] and PACAP38 receptors are present in the cranial ganglia with perivascular nerve projections [27]. Furthermore, PACAP27 and PACAP38 concentrations increased in the TNC of rats after electrical or chemical stimulation of the trigeminovascular system [44]. In the rat, the administration of a PAC1 receptor antibody, AMG 301, inhibited the stimulus-evoked nociceptive activity in the TNC [45]. PACAP38 but not VIP activated trigeminocervical neurons [46]. In experimental animal models, intrathecal administration of capsaicin resulted in high levels of PACAP27 and PACAP38 in the cerebrospinal fluid, supporting the hypothesis of a central effect of PACAP38 [47]. Intrathecal administration of PACAP6-38, a PAC1 antagonist, reduced mechanical and thermal hyperalgesia [48]. In another study, the same PAC1 antagonist increased dynorphin release, while PACAP38 administration decreased dynorphin release [49]. In accordance with these results, PAC1 receptor knockout mice showed reduced nociceptive responses to different stimuli [50].

2.2. Targeting PACAP in migraine prophylaxis

The exact mechanisms of PACAP38-induced migraine are still unknown; however, a lot of evidence suggests that this polypeptide plays a key role on migraine. Therefore, inhibition of PACAP38 could be an effective option for migraine treatment, so two strategies were attempted: blocking the PAC1 receptor or PACAP38 itself.

Using a monoclonal antibody against the PAC1 receptor, AMG 301, a phase 2a, randomized, double-blind, placebo-controlled, 3-arm, and parallel group clinical trial (NCT03238781) was completed to evaluate efficacy and safety in migraine prophylaxis during a 12 week double-blind treatment period. In particular, the effect of AMG 301 on the change from the baseline period in monthly migraine days was compared to placebo. Participants were randomized 4:3:3 to placebo, AMG 301 210 mg Q4W or AMG 301 420 mg Q2W, respectively. From the results reported in ClinicalTrials.gov, no statistically significant differences were found between AMG 301 and placebo for primary and secondary outcomes. The most common adverse events were nasopharyngitis (9.62% and 6.86% for 210 mg and 420 mg, respectively, versus 9.49% for placebo), fatigue (4.81% and 8.82% for 210 mg and 420 mg, respectively, versus 5.84% for placebo), influenza (4.81% and 5.88% for 210 mg and 420 mg, respectively, versus 3.65% for placebo), and constipation (3.85% and 5.88% for

210 mg and 420 mg, respectively, versus 0% for placebo). Only 3 patients receiving AMG 301 reported severe treatment-emergent adverse events (versus 3 receiving placebo); no fatal events were reported.

The antagonist properties of ALD1910, a monoclonal antibody 4000-fold more selective for PACAP38 and PACAP27 than VIP, were tested in an umbellulone-induced rat model of headache. Umbellulone acts on the TRPA1 receptor, inducing neurogenic vasodilation (clinically evident as an increase in the animal's facial temperature) and parasympathetic lacrimation. ALD1910 led to a dose-dependent inhibition of umbellulone-induced neurogenic vasodilatation and parasympathetic lacrimation [51]. At last, a first-in-human, randomized, double-blind, and placebo-controlled study in a healthy population is ongoing to determine the safety and tolerability of ALD1910 (NCT04197349).

3. Conclusion

Intravenous infusion of PACAP38 induces migraine in healthy volunteers and triggers delayed migraine-like headaches in most migraine patients [30,34,52]. This effect and PACAP38's role in experimental models of neurogenic inflammation indirectly demonstrated that it is involved in activating the trigeminovascular system. This polypeptide is the most represented PACAP isoform in the brain regions associated with migraine [52]. Based on this evidence, treatments with monoclonal antibodies targeting PACAP or its receptor are being developed. These include ALD1910, developed by Alder BioPharmaceuticals, which targets the peptide and began a first-in-human, randomized, double-blind, and placebo-controlled study (NCT04197349) in a healthy population in October 2019 and AMG 301, developed by Amgen Inc., which targets the PAC1 receptor [20,51]. Recently, AMG 301 completed a phase 2a controlled trial (NCT03238781) in migraine prevention, whose results were unfortunately unsatisfactory.

Despite the initial failure, the potential of anti-PACAP antibodies in migraine prophylaxis remains high. Theoretically, if these antibodies block the activation of the trigeminovascular system, they will completely prevent the onset of migraine attacks. In addition, monoclonal antibodies should not exhibit pharmacokinetic drug-drug interactions, which is optimal for migraine prophylaxis, as migraine patients are subject to polypharmacy [53]. However, differences in the release of PACAP and CGRP induced by 60 mM potassium or capsaicin were recently examined in a rat migraine model. PACAP has been observed to be released only within the TG, but not from sensory fibers in the dura mater, as occurred for CGRP. These data suggest that anti-PACAP antibodies, unable to penetrate BEE, may not reach their central targets and thus could be ineffective in treating migraine [54]. In any case, bearing in mind that PACAP has vital functions, the risk/benefit ratio of anti-PACAP antibodies must be carefully studied.

At the time of this review, there is abundant evidence of PACAP's ability to cause migraine, but there is little proof that blocking PACAP or its PAC1 receptor can prevent migraine. In some ways, PACAP research resembles nitrous oxide (NO)

studies. NO donor drugs cause migraine. For example, the administration of nitroglycerin, a NO donor, has been widely used as a human experimental model of migraine [55]. However, selective NOS inhibitors were found to be ineffective in acute and preventive treatment of migraine [55,56]. Now, the opposite hypothesis is being tested, i.e., that the increase in local and systemic NO levels may have therapeutic potential in preventive treatment for episodic migraine (NCT03488563). However, all the studies carried out are helpful to increase knowledge on the pathophysiology of migraine, even if they do not lead to the development of drugs for clinical use.

4. Expert opinion

The idea of blocking PACAP or its specific receptor PAC1 to prevent migraine follows the winning path that led to the development of CGRP antagonists but has not yet produced the same success. Unfortunately, the results of the phase 2a trial (NCT03238781, reported in clinicaltrials.gov and not yet published) with the anti-PAC1 monoclonal antibody, AMG 301, in migraine prophylaxis are unsatisfactory. To explain this failure, Hoffman et al. [45] suggested that AMG 301 could be useful specially in patients who have autonomic symptoms associated with the migraine attack, while in this trial the stratification of the patients based on this characteristic was not performed. The poor results cast doubts on the role of the PAC1 receptor as a mediator of PACAP's migraine provocation action. Speculatively, other reasons for failure may have been an insufficient or incomplete occupation of PAC1 receptors by AMG 301 on all the different receptor isoforms or an increased activation of VPAC1 and VPAC2 receptors against the blockade of PAC1, or even the activation of the PAC1 receptor by VIP.

In theory the block of endogenous PACAP should not allow the activation of migraine attacks. ALD1910 is 4000 times more selective for PACAP38 and 27 than for VIP and its antagonist activity was dose-dependent in an *in vivo* animal model [51]. However, VIP and PACAP have several shared functions [57]. Intravenous infusion of VIP prolonged for 2 hours induced delayed headache and extracranial vasodilation in healthy volunteers [58]. It cannot be excluded that VIP

may replace PACAP on all 3 of its receptors, equally causing the migraine attack.

Despite the beginning of the clinical phase, research on PACAP in migraine still presents areas of uncertainty that should be clarified. We are still far from understanding the full range of mechanisms that could involve PACAP and its receptors in triggering, evolving, and extinguishing the migraine attack. However, it bodes well that research is focusing more than ever on the molecular mechanisms of these processes [59]. First, the role of the PAC1 receptor as a mediator of PACAP's ability to cause migraines is uncertain. Second, the functions in migraine of VPAC1 and VPAC2 receptors, which have similar affinities for PACAP and VIP, have not yet been studied in depth. However, all PACAP/VIP receptors are present in sensory neurons and vascular smooth muscle related to the trigeminovascular system [60]. Furthermore, although the substances that cause migraine are normally vasodilators and both PACAP and VIP are vasoactive peptides, dilation of arterial vessels is not the only factor behind migraine [61]. In order to trigger the migraine attack, activation of the trigeminovascular system is believed to be essential [62,63]. Endogenous PACAP was proposed just as the mediator of this action, whose mechanisms, however, have not yet been clarified [59]. Besides, the existence of specific PACAP signaling circuits involved in the induction of migraine is still being investigated [10].

Surprisingly, we have found no published attempts to predict the possible risks to which migraine patients could be exposed due to long-term blockade of endogenous PACAP or its specific PAC1 receptor. Yet, PACAP is a pleiotropic neuropeptide that has critical roles in the regulation of stress responses [64] and a plethora of vital functions (some of those recently studied are listed in Table 1). It influences neurotransmission and neuromodulation and has an inhibitory effect on neurogenic inflammation and a protective one in neuronal ischemic lesions [65,66]. PACAP intervenes in the regulatory mechanisms of the gastrointestinal, cardiovascular, reproductive, and respiratory systems and in nociceptive processes [67]. Finally, the use of analogues or long-lasting PACAP agonists is advocated for the treatment of various disorders (Table 2), rather than blockade.

Table 1. Some of the PACAP functions recently explored.

Target	Function	References
Reproduction	PACAP modulates reproductive functions and intervenes in the regulation of fertility. Mortality is high among PAC1 or PACAP knockout mice and reaches 60% at one month of age. These mice have severe metabolic disorders and reduced mating and maternal behaviors.	Köves K, Szabó E, Kántor O, et al. Current state of understanding of the role of PACAP in the hypothalamo-hypophyseal gonadotropin functions of mammals. <i>Front Endocrinol.</i> 2020;11:88.
Thermogenesis	PACAP plays a fundamental role in thermogenesis for the defense of body temperature	Cline DL, Short LI, Forster MAM, et al. Adipose tissue expression of PACAP, VIP, and their receptors in response to cold stress. <i>J Mol Neurosci.</i> 2019;68:427–438.
Obesity	Leptin and PACAP collaborate together for maintaining normal energy homeostasis and body weight.	Hurley MM, Anderson EM, Chen C, et al. Acute blockade of PACAP-dependent activity in the ventromedial nucleus of the hypothalamus disrupts leptin-induced behavioral and molecular changes in rats. <i>Neuroendocrinology.</i> 2020;110:271–281.
Eating	PACAP suppresses the intake of food, nicotine and alcohol.	Gargiulo AT, Curtis GR, Barson JR. Pleiotropic pituitary adenylate cyclase-activating polypeptide (PACAP): novel insights into the role of PACAP in eating and drug intake. <i>Brain Research.</i> 2020;1729:146,626.

Table 2. Potential therapeutic uses of PACAP.

Target and function	Therapeutic use	References
Neuroprotection PACAP has important neuroprotective effects in several models of neuronal injury and appears to be implicated in acute and chronic neuronal pathological conditions. The decline of this endogenous peptide may indicate neuronal degeneration and accelerated age-related degeneration.	PACAP represents a good target for the future development of drugs to treat ischemic and neurodegenerative diseases	Rivnyak A, Kiss P, Tamas A, et al. Review on PACAP-induced transcriptomic and proteomic changes in neuronal development and repair. <i>Int J Mol Sci.</i> 2018;19. Reglodi D, Atlasz T, Szabo E, et al. PACAP deficiency as a model of aging. <i>Geroscience.</i> 2018;40:437–452.
Retina PACAP (and its receptors) is found in the eye tissues where it performs protective activity.	PACAP, in the form of eye drops, has been proposed in the treatment of corneal and retinal diseases.	Kovacs AK, Atlasz T, Werling D, et al. Stability test of PACAP in eye drops. <i>J Mol Neurosci.</i> Available from: http://link.springer.com/10.1007/s12031-020-01532-9 .
Cartilage PACAP plays an essential role in osteogenesis and normal development of cartilage.	PACAP has therapeutic potential in the treatment of degenerative cartilage diseases such as osteoarthritis	Lauretta G, Ravalli S, Szychlinska MA, et al. Current knowledge of pituitary adenylate cyclase activating polypeptide (PACAP) in articular cartilage. <i>Histol Histopathol.</i> 2020;18,233.
Liver transplantation PACAP is cytoprotective and promotes liver transplant survival.	PACAP has been proposed as a new treatment in orthotopic liver transplantation	Liu Y, Lu T, Zhang C, et al. Pituitary adenylate cyclase-activating polypeptides prevent hepatocyte damage by promoting yes-associated protein in liver ischemia-reperfusion injury. <i>Transplantation.</i> 2019;103:1639–1648.

In our opinion, literature is also lacking regarding the examination of the potential advantages of anti-PACAP antibodies, compared to those of antibodies against CGRP or its receptor. Indeed, the relationship between CGRP and PACAP in migraine remains undefined, even if PACAP is co-localized with CGRP in the TG and in the TNC [59]. Both PACAP 38 and CGRP induce migraine attacks, although not in all patients [43]. Considering that PACAP and CGRP have overlapping effects, it is not clear why blocking PACAP should be more effective or advantageous compared to the antagonism of CGRP. It has been suggested that PACAP38 could cause migraine independently of CGRP [68], as intravenous infusion of PACAP38 does not cause an increase in CGRP blood levels [43]. On this basis, anti-PACAP antibodies could perhaps be useful to patients who do not respond to anti CGRP drugs [20].

We think that the first task of research today is to clarify all the still undefined aspects of the role of PACAP in migraine and to allow clinical development to proceed. If anti-PACAP antibodies are available for therapeutic use, it will be interesting to study whether there are differences, and which, between the blockade of the PAC1 receptor and that of PACAP. Furthermore, anti-PACAP antibodies should compete with anti-CGRP drugs, which, in the meantime, are proving effective and safe in the short term, also in the real world. However, their long-term safety is still under evaluation [69]. On the other hand, if anti-PACAP antibodies are shown to be ineffective and risky for prevention, the development of an antagonist of the peptide or its PAC1 receptor for acute treatment may be attempted. A temporary blockage of PACAP or PAC1 receptor could perhaps prove safe and effective.

Finally, to obtain results easily applicable to clinic, in our opinion it is necessary to find biomarkers (through neuroimaging, proteomics, metabolomics, etc.) to be integrated into clinical trials that can help identify the right migraine patients for one drug in the early phase of development. If all efforts of researchers and clinicians are completed, a 'precision medicine' approach [70] may therefore be possible in the future, for the benefit of every migraine patient.

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ORCID

Cecilia Rustichelli  <http://orcid.org/0000-0001-8917-9980>
 Flavia Lo Castro  <http://orcid.org/0000-0002-8572-3421>
 Carlo Baraldi  <http://orcid.org/0000-0001-8432-1888>
 Anna Ferrari  <http://orcid.org/0000-0001-9536-3995>

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