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REVIEW

Migraine pathways and the identification of novel therapeutic targets

Innocenzo Rainero[a], Fausto Roveta[b], Alessandro Vacca[c], Cecilia Noviello[b] and Elisa Rubino[c]

[a]Headache Center Department of Neuroscience "Rita Levi Montalcini", University of Torino, Torino, Italy; [b]Department of Neuroscience, University of Torino, Torino, Italy; [c]Department of Neuroscience and Mental Health, Città della Salute e della Scienza di Torino, Torino, Italy

ABSTRACT

Introduction: Migraine is a chronic neurovascular disorder characterized by recurrent headache attacks associated with neurological and autonomic symptoms. The pathophysiological mechanisms of the disease are extremely complex, involving hypothalamic and trigeminovascular activation, cortical spreading depression, release of pro-inflammatory peptides, peripheral and central sensitization. The underlying cellular and molecular mechanisms have been scarcely investigated. Recently, genetic studies have suggested that different metabolic pathways could be involved in the pathogenesis of migraine.

Areas covered: This review focuses on cellular and molecular mechanisms involved in migraine, suggesting a role for circadian clocks, ion channels, synaptic plasticity, vascular factors, ion metal homeostasis, and impaired glucose metabolism in the pathogenesis of the disease. Accordingly, the article proposes new therapeutic targets that may be of particular relevance for disease prevention.

Expert opinion: Several complex molecular mechanisms are involved in setting the genetic threshold for migraine and the pathogenesis of headache attacks. Most promising new therapeutic targets are the modulation of hypothalamic activity and ion channels involved in pain transmission. Further studies in animals and humans are necessary to enhance the elucidation of the molecular mechanisms of migraine and open new avenues for disease prevention.

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Migraine; aura; circadian clocks; hypothalamus; ion channels; metal ion homeostasis; glucose metabolism; therapeutic target; PACAP1-38; TRPM8

1. Introduction

Migraine is a chronic neurovascular disorder characterized by recurrent headache attacks of moderate to severe intensity, associated with autonomic and neurologic symptoms. The diagnosis of the disease relies only on the clinical criteria defined by the International Classification of Headache Disorders – 3rd Edition (ICHD-3) [1]. These include the presence of pulsating headache attacks, lasting 4–72 h, aggravated by routine physical activity and associated with nausea and/or vomiting, photophobia, and phonophobia. In addition, in approximately one-third of migraineurs, headache attacks are preceded or accompanied by the aura phenomenon, consisting of fully reversible focal neurologic symptoms as visual disturbances, sensory deficits, or other central nervous system (CNS) [2]. According to ICHD-3 criteria, migraine is classified into two major subtypes: migraine without aura (MO) and migraine with aura (MA). At present, it is unclear whether MA and MO belong to the same spectrum of illness or are two distinct disorders [3,4].

Migraine is a highly heterogeneous disease. A significant gender effect has been demonstrated in the disease, with a female to male ratio of 3:1 that significantly varies during lifetime [5]. Age at onset and duration of the disease, duration of the migraine attacks as well as accompanying symptoms diversify greatly between patients and even in the same patient in different attacks [6,7]. Generally, migraine is an episodic disorder (EM) but, each year, approximately 3% of patients convert into chronic migraine (CM), a condition characterized by headache attacks occurring on 15 or more days/month for more than 3 months [1]. CM develops after a slow increase in headache frequency over months to years, a process termed 'migraine transformation,' and is often associated with overuse of acute medications [8]. Finally, in comparison with the general population, several medical conditions are more frequent in migraine patients. Migraine comorbidities include psychiatric, neurologic, cardiovascular, allergic, and endocrine diseases [9–14]. The development of one or more of these comorbidities significantly modifies the clinical characteristics of the disease.

In recent years, the prevalence and incidence of migraine have been intensively investigated. In Western Countries, approximately 12% of the adult population (18% of women and 6% of men) fulfilled the criteria for EM, while 1-2% for CM [7,15,16]. The incidence rate for EM is approximately 10 per 1000 person-years, with a woman to man ratio of 6 to 1. Migraine is a highly burdensome condition for patients, families, and society. An analysis of the 2016 Global Burden of Disease data estimated that the disease caused 45.1 million years lived with disability in that year [17]. Migraine is the second most disabling condition after low back pain. The burden of migraine occurs not only during the *ictal* phase (headache attacks) of the disease but also in the *interictal* period. All the symptoms of the migraine attacks (nausea,

CONTACT Innocenzo Rainero innocenzo.rainero@unito.it Neurology Headache Center Department of Neuroscience 'Rita Levi Montalcini', University of Torino, Torino, Italy

Article Highlights

- Genetic factors are key players in positioning the threshold for migraine. Mendelian forms of migraine are very rare while the more common forms are transmitted as oligogenic or polygenic complex traits.
- Genetic studies showed that different metabolic pathways may be involved in the predisposition to the disease and in headache attacks.
- Hypothalamic activation has a significant role in the pathogenesis of the migraine attack. Further development of monoclonal antibodies that target PACAP1-38 and its receptor may be valuable in migraine therapy.
- Modulation of ion channels that are involved in pain transmission, e.g., TRESK and TRPM8, may offer new opportunities in migraine prophylaxis.
- Animal and human studies are necessary for the elucidation of the complex molecular mechanism of migraine and potential therapeutic targets.

This box summarizes the key points contained in the article.

vomiting, and sensitivity to environmental stimuli) significantly limit the patient's functioning, while in the interictal phase, the burden of the disease is related to the anxiety, fear of the next attack, and difficulty in planning new events [18]. The costs of migraine, due to both direct (utilization of health-care resources) and indirect costs (loss of productivity), are enormous and generate a significant societal burden [19,20].

2. Pathophysiological mechanisms of migraine

Migraine pathophysiological mechanisms are very complex and still not completely understood [21,22]. First of all, it is necessary to clearly distinguish the mechanisms involved in the interictal phase of the disease ('the migrainous brain') from the pathophysiological mechanisms of the migraine attack.

Several neurophysiological studies have shown that the migraine brain, in the interictal phase of the disease, is characterized by general neuronal hyperexcitability. The responses to a wide range of stimuli, including visual, somatosensory, and auditory stimuli, are significantly increased in migraineurs as compared to normal controls [23]. The lack of habituation in response to repetitive stimulation is the main neurophysiological trait of the disease [24]. Also functional imaging studies have shown that, during the interictal phase, the brain of migraine patients is hyper-responsive to different stimuli [25].

Genetic factors play a major role in setting the threshold for migraine neuronal hyperexcitability. Mutations in several genes coding for ion channels, like the calcium voltage-gated channel subunit alpha1A (*CACNA1A*), sodium voltage-gated channel alpha subunit 1 *(SCN1A*), potassium two-pore domain channel subfamily K member 5 *(KCNK5*), potassium two-pore domain channel subfamily K member 18 *(KCNK18*), and transient receptor potential cation channel subfamily receptor M member 8 (*TRPM8)* genes, have been associated with the migraine phenotype [26]. Therefore, migraine may be included in the large group of neurological ion channelopathies.

The migraine attack characterizes the ictal phase of the disease. This is a complex and long-lasting neurobiological phenomenon due to the coexistence of disturbances in sensory, affective, cognitive, and autonomic functions. These clinical features suggest the involvement of several brain networks. Traditionally, the migraine attack has been divided into four different phases: the prodrome, the aura, the headache phase, and the postdrome [27]. The symptoms of the *prodrome* phase of the migraine attacks include mood changes, fatigue, yawning, food cravings, and hypersensitivity for external stimuli [28]. Functional neuroimaging studies, using both positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), provided clear evidence of the involvement of the posterolateral hypothalamus during the prodromal phase of migraine attack. In addition, networks connecting the hypothalamus with brain areas related to autonomic function are significantly impaired in all the phase of the attacks, a possible explanation of altered autonomic functions observed during the crisis [29,30].

The *aura* phase of the migraine attack is currently explained by the phenomenon of the cortical spreading depression (CSD). This is characterized by a slowly (3 mm/min) propagating wave of depolarization in neuronal and glial cell membranes that is followed by inhibition of cortical activity up to 30 min [31]. This wave of spreading depression is also associated with a wave of hyperemia, followed by a prolonged phase of cortical oligemia [32]. CSD is associated with a large efflux of potassium ion (K^+) from the neurons to the interstitial spaces, influx of sodium ion (Na^+) and calcium ion (Ca^{2+}), and release of glutamate [33].

The activation of the trigeminovascular system is the better explanation of the characteristic pulsating *headache* phase. This pathway normally conveys nociceptive information from the meninges to the central areas of the brain, including several brainstem nuclei, hypothalamus, thalamus, and cortex [23]. Antidromic activation of the trigeminovascular system promotes the release of proinflammatory peptides, such as CGRP and pituitary adenylate cyclase-activating polypeptide 1–38 (PACAP1-38) [34]. CGRP is a 37 amino acid neuropeptide encoded by the calcitonin (*CALCA*) gene that plays a major role in migraine pathogenesis, being a potent vasodilator and an enhancer of glutamatergic transmission [35]. During the headache phase of the migraine attack both peripheral and central sensitization occur, explaining symptoms like exacerbation of pain by routine physical activity and allodynia [36].

The *postdrome* is the least studied and hence the least understood phase of the acute migraine attack. The commonest symptoms are tiredness, cognitive difficulties, 'hangover,' gastrointestinal symptoms, depressed or euphoric mood, and weakness [37]. Functional imaging showed a widespread reduction in cerebral blood flow in the postdrome, possibly explaining the multitudes of symptoms reported by patients [38].

3. Genetics of migraine

Since the nineteenth century, migraine has been recognized to 'runs in families' and considered to be a strongly heritable disorder [39]. A large number of population studies, as well as twin studies, have demonstrated that migraine has a genetic basis [40,41]. Several families with autosomal dominant segregation of the migraine phenotype, often with atypical features,

have been described in literature [42]. Monozygotic twins reared together or apart, show a significantly higher concordance rate for the disease in comparison with dizygotic twins [43]. First-degree relatives of probands with migraine have an increased risk for the disease in comparison with the general population [40]. However, monogenic forms of migraine are rare, and the more common form of the disease is transmitted as polygenic complex traits, due to the contribution of variants with small effect at many genetic loci. In addition, both MA and MO are characterized by a significant genetic heterogeneity with a large phenotypic variability [26].

In the last two decades, molecular genetic studies have provided considerable insights into the molecular mechanisms of migraine, suggesting new therapeutic targets. First of all, Familial Hemiplegic Migraine (FHM), a rare MA subtype characterized by an autosomal dominant transmission of the phenotype and presence of transient motor deficits, has been associated with mutations in three different genes, *CACNA1A, ATP1A2*, and *SCN1A*, coding for protein linked to ion channels functions [44–46]. In addition, investigating families with rare, monogenic forms of migraine, pathogenic variants in other genes, as *KCNK18, PRRT2, PNKD, SLC2A1, SLC1A3*, and *SLC4A4* have been demonstrated [47,48]. In the more common form of migraine, candidate gene association studies showed that more than 200 genetic variants in approximately 100 different genes may be associated or influence the clinical characteristics of the disease [49]. However, these studies often report conflicting results and have been rarely replicated. Finally, several genome-wide association studies (GWAS) provided evidence that numerous single-nucleotide polymorphisms (SNPs), and related genes, are significantly associated with migraine and its clinical variants. The most recent meta-analysis combined the data from 22 GWAS, comprising 59,674 migraine cases from clinic- and population-based collections as well as 316,078 controls [50]. This study demonstrated that 44 independent SNPs at 38 distinct genomic loci are significantly associated with migraine, providing new molecular insight into the molecular pathways of migraine.

Recent studies tried to relate genetic variants reported in GWAS to common molecular pathways in order to explain the basic biology of this disorder [26,40]. Accordingly, metabolic pathways related to circadian clocks, ion channel, cellular and synaptic mechanisms, vascular mechanisms, metal ion homeostasis, and glucose metabolism have been suggested as a potential explanation of migraine susceptibility. Following these suggestions, our review discusses the role of these molecular pathways in discovering new potential therapeutic targets for this frequent and disabling disease.

4. Circadian clocks

In mammals, the circadian timing system consists of a central brain clock, located in the suprachiasmatic nucleus (SCN) of the hypothalamus, and several peripheral clocks in other brain regions and in different tissues throughout the body. The SCN network, through the retinohypothalamic tract, is connected with the ganglion cells of the retina and oscillations in environmental light synchronizes the circadian rhythm of the SCN with the environmental 24 h rhythm. The timing signal from the SCN is transmitted via both neural and hormonal signals as well as body temperature to the peripheral clocks [51].

The molecular mechanism of the central and peripheral clocks is based on transcriptional-translational feedback loops, which are present in almost every cell of the human body. In neurons within the SCN, but also in peripheral clocks, Clock and Bmal1 activate the expression of many targets, including Period (Per1, 2, and 3) and Cryptochromes (Cry1 and Cry2) that act as negative regulators. As Per and Cry proteins increase in concentrations, they multimerize, enter into the nucleus, and bind to the heterodimeric Clock and Bmal1 complex, inhibiting their transcriptional activity (see Figure 1). This mechanism, that is cell-autonomous, generates a 24-h cycle [52]. This machinery is functional in all mammalian tissues and influence many physiological functions.

Migraine is considered a chronobiological disorder, with a characteristic periodicity [53]. Often, migraine attacks present a morning onset (6 to 9 am), though acute headache episodes may occur along all day. In addition, a late-night peak is present, particularly with advancing age [54,55]. Therefore, hypothalamic peptides involved in the regulation of the circadian clock may have a role in migraine pathogenesis. Recently, increased interest has been devoted to the study of two peptides, pituitary adenylate cyclase-activating peptide 1–38 (PACAP1-38) and casein kinase 1 *delta* (CKIδ), in migraine pathogenesis [56,57].

PACAP1-38 is a multifunctional vasodilatory peptide that shows several similarities with CGRP. It is encoded by *ADCYAP1* gene, which expresses two forms containing either 27 or 38 amino acids with PACAP1-38 being the more prevalent. PACAP1-38 binds to three different G-protein coupled receptors, which have an affinity for vasoactive intestinal peptide (VIP) as well. It is expressed throughout the CNS as well as in peripheral organs and glands. Within the CNS, PACAP1-38 has roles in neurodevelopment, neuroprotection, neuromodulation, neurogenic inflammation, and nociception [58,59]. PACAP1-38 plasma levels are elevated during a migraine attack while interictal PACAP1-38 concentrations are significantly lower in comparison to healthy subjects [60]. In addition, intravenous injection of PACAP1-38 in migraineurs is able to induce migraine-like headache attacks [61].

The CKIδ protein is a central component of the circadian clock and is encoded by the *CSNK1D* gene. CKIδ is a ubiquitous serine-threonine kinase that phosphorylates the circadian clock protein PER2, as well as other proteins involved in brain signaling [52]. In two large independent pedigrees, a co-segregation between mutations in the *CSNK1D* gene and a complex phenotype characterized by both familial advanced sleep phase syndrome and MA was found [57,62]. It was suggested that the mutant gene product CK1δ may cause vascular dysfunction through abnormal astrocytic signaling in conjunction with CSD. Intriguingly, experimental animals carrying a transgene with the human CKIδ-T44A mutation displayed a reduced threshold for CSD [62]. These data further suggest a role for the hypothalamus in migraine susceptibility and underline the correlation between migraine and sleep disorders.

Traditionally, therapeutic interventions targeting the circadian clock system in migraine evaluated the use of melatonin as regulator of the circadian rhythms. However, the current literature regarding the use of melatonin for migraine

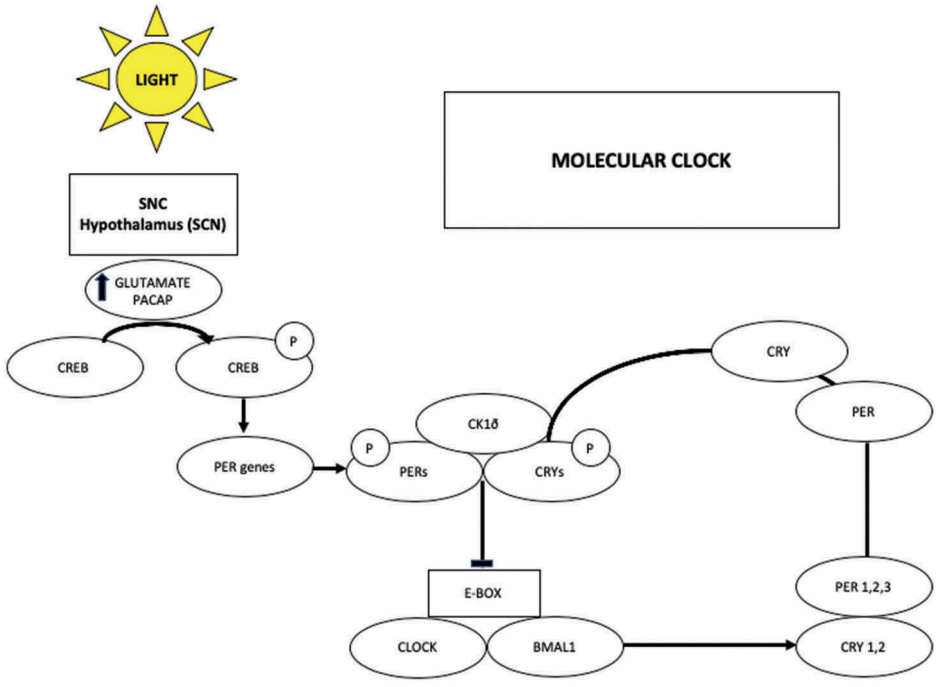

Figure 1. Simplified molecular dynamics of the circadian clock.

In master neurons within the suprachiasmatic nucleus (SCN), and downstream in extra-SCN and peripheral clocks, the positive transcriptional activators Clock and Bmal1 bind to E-box motifs and activate the expression of many targets, including their own negative regulators, Period (Per1, 2, and 3), and Cryptochromes (Cry1 and Cry2). As the negative feedback proteins Per and Cry increase in abundance, they interact with the serine/threonine kinase casein kinase-1 (CKIδ), as well as with other epigenetic regulators (not shown in this figure), multimerize, enter into the nucleus, and bind to the heterodimeric Clock and Bmal1 complex to inhibit their transcriptional activity. Pacemaker neurons within the SCN receive photic input from retino-hypothalamic tract that release glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP), which leads to the phosphorylation of cAMP response element (CRE)-binding (CREB) protein and activation of immediate early genes and expression of Per genes. Pacemaker neurons of the SCN are thus responsible for synchronizing downstream extra-SCN and peripheral clocks to the external light–dark cycle.

prophylaxis is limited [63,64]. In addition, alternative therapeutic strategies, like light therapy, may be of relevance for migraine prophylaxis. Intriguingly, antibodies against PACAP1-38 (ALD 1910) and PAC_1 receptor (AMG 301) have been developed, with AMG 301 already in Phase II clinical trials [65]. No results have been published so far, but in preliminary studies, AMG 301 has shown to be effective in controlling migraine attacks. At present, the potential side effects of long-term blockade of the PACAP1-38 pathway are unknown. However, it is important to investigate whether these antibodies will indeed represent a therapeutic advantage for the patients that do not respond to the CGRP antibodies.

5. Ion channels

In the last two decades, the notion that migraine may be related to neuronal channelopathies, a group of genetically and phenotypically heterogeneous neurologic disorders that result from defects in ion-channel function, has gained substantial support [66]. The discovery of the FHM genes, showing co-segregation of mutations in genes coding for ion channels with the clinical phenotype, strongly supported this idea. However, even if ion channel dysfunction plays a pivotal role in migraine development, mutations in a single ion channel often do not explain the full pathogenesis of the disease. Recently, mutations in two new genes coding for ion channels, *KCNK18* and *TPRM8*, have been repeatedly associated with the disease. These two genes are, at present, interesting candidate for the development of new strategies in migraine therapeutics.

The *KCNK18* gene is located on 10q25.3 and encodes the TWIK-related spinal cord potassium (TRESK) channel, which belongs to the potassium two-pore (K2P) family [67]. This channel is composed of four transmembrane segments and two extracellular reentrant pore loops. TRESK is expressed in the CNS, and in normal conditions leaks currents regulating neuronal resting membrane potentials. In experimental animals, knock-out animals for *KCNK18* did not show a gross anatomical or behavioral phenotype [68]. However, the gene ablation alters the duration of action potential in the dorsal root ganglion, a key structure in migraine pathogenesis. In addition, in inflammatory conditions, *KCNK18* can reduce the neuronal excitability, mainly to its coupling to the histamine H_1 receptor [69]. A large Canadian family, showing segregation of a *KCNK18* mutation with a typical MA phenotype was described in 2010 [47,70]. The observed mutation causes a premature truncation in the first transmembrane region, resulting in a nonfunctional protein. Additional screenings for *KCNK18* mutations in European and Australian populations identified a large number of other genetic missense variants both in MA and in MO. Some of these variants did not show a functional effect, whereas others caused a damaging effect on channel activity [71]. However, some genetic variants were also found in healthy controls, indicating that the presence of a single mutation is not sufficient to determine whether a subject present migraine. At present, the precise role of the *KCNK18* in migraine etiopathogenesis is still a matter of debate.

Several, recent GWAS have found a significant correlation between migraine incidence and SNPs located near the

Transient receptor potential melastatin 8 (TRPM8) coding region [72,73]. TRPM8 belongs to transient receptor potential (TRP) superfamily, a large family of nonselective cation channels that can be activated by a wide variety of stimuli, including changes in temperature, osmolarity, and pH. Activation of TRPs allows the influx of Ca^{2+} and Na^{+}, resulting in membrane depolarization as well as the activation of second messenger signaling cascades. TRP channels participate in the sensory encoding of pain under both normal and disease states. The TRPM8 gene is located at 2q37.1 region and codes for a channel protein, expressed in sensory neurons, that is activated by cold temperatures and cooling agents, such as menthol [74]. Studies in experimental animals showed that activation of TRPM8 causes migraine-like behaviors that can be blocked with TRPM8 antagonists. Pre-treatment with sumatriptan, a drug commonly used to treat migraines, prevents these migraine-like behaviors, which strongly implicates a role for TRPM8 in migraine generation. Intriguingly, activation of TRP channels can elicit CGRP release [75].

In the last few years, antagonists of TRPM8 have been investigated as new drugs for chronic pain, migraine, or inflammation. Both TRPM8 agonists and antagonists may be potential therapeutics, depending on how migraine is triggered in individual patients. At present, different drugs that modulate TRPM8 channels are under investigation in patients with migraine [76,77].

6. Synaptic plasticity

There is a long-standing debate on whether migraine is associated with structural brain lesions. Several neuroimaging studies suggested that structural abnormalities, like volumetric changes in gray and white matter regions, white matter abnormalities and infarct-like lesions are more common in migraineurs than in controls [78]. Recently, molecular genetic studies provided preliminary suggestions to explain these microstructural alterations in migraine.

Hemiplegic migraine and migraine with aura may be associated with the paroxysmal dyskinesias, a group of intermittent movement disorders often caused by mutations in genes related to the cell membrane and synaptic function. Two genes, the proline-rich transmembrane protein 2 (*PRRT2*) and the paroxysmal nonkinesiogenic dyskinesia (*PNKD*) gene, have been recently investigated for their potential correlation with migraine pathogenesis [26,79].

The *PRRT2* gene encodes a presynaptic transmembrane protein, PRRT2, that negatively regulates several proteins of the SNARE complex [80]. Recently, an important role of PRRT2 in synapse development, regulation of calcium channels in glutamatergic neurons and neurotransmitter release has emerged [81]. More than one hundred of missense and, most frequently, loss of function truncating mutations in *PRRT2* gene have been reported worldwide. The phenotypes associated with *PRRT2* mutations included, in addition to paroxysmal dyskinesia and ataxia, a high frequency of migraine and hemiplegic migraine [82,83]. In experimental animals, PRRT2 silencing significantly decreases the number of excitatory synapses and selectively impairs Ca(2+)-dependent neurotransmitter release [84].

In addition, PRRT2 affects glutamate receptor activity via interactions with glutamate receptor gene 1.

The *PNKD* gene is located at 2q35 and contains 12 exons. There are at least three isoforms of this protein of varying lengths (long, medium, and short), that can be produced by alternative splicing [85]. These isoforms also differ in their tissue-specific expression and subcellular localization: the long isoform is expressed only in CNS, whereas remaining isoforms are ubiquitously expressed. PNKD protein interacts with several synaptic active zone proteins, and mutant proteins are less effective at inhibiting exocytosis, resulting in increased neurotransmitter release [86]. To date, little is known about how the disruption of PRRT2 and PKND proteins may contribute to migraine pathogenesis. However, studies in transgenic mice showed increased glutamatergic neurotransmission and cerebral hyperexcitability, indicating that the lack of neurotransmitter release regulation could contribute to the migraine phenotype [87].

Brain structure changes in migraine could potentially identify subgroups of patients with specific therapeutic needs and prognoses. In hemiplegic migraine cases with *PRRT2* mutations, some benefit has been observed with carbamazepine, a sodium channel blocker antiepileptic drug [88]. However, additional studies are needed in order to better investigate drugs that may modulate structural and synaptic plasticity in the disease.

7. Vascular mechanisms

One of the main findings of GWAS in migraine is that SNPs in genes that are expressed in arterial tissue are significantly associated with the disease. These findings suggest that vascular dysfunction plays a major role in migraine pathophysiology and is not only the consequence of neuronal dysfunction. In addition, these data provide additional support to the comorbidity between migraine, stroke, and cardiovascular diseases as the same genetic factors are involved in their genetic predisposition [50,89].

Several GWAS found an association between the intronic SNP rs9349379 at PHACTR1 locus (6p24) with migraine phenotype but also with coronary artery disease, cervical artery dissection, fibro-muscular dysplasia, and hypertension [90]. Intriguingly, this SNP influences the expression of the *EDN1* gene, located 600 kb upstream of PHACTR1, that codes for endothelin-1 (ET-1). ET-1 is a 21-amino acid long peptide produced by endothelial cells, vascular smooth muscle cells, and macrophages. ET-1 is a potent, longest-lasting vasoconstrictor in humans. In addition, a role for the peptide in neural crest development and regulation of salt balance has been demonstrated. Therefore, drugs able to modulate ET-1 secretion as well as blocking ET-1 receptors could be investigated in the prophylaxis of migraine.

8. Metal ion homeostasis

An unexpected hypothesis that emerged from GWAS in migraine is that metal ion homeostasis might contribute to migraine susceptibility. Several genes with such function, like

PRDM16, TGFBR2, REST, FHL5, NRP1, MMPED2, LRP1, ZCCHC14, RNF213, and *JAG1*, are significantly associated with the disease [89]. Metal ions participate in numerous metabolic functions in living cells, and an aberration in metal ion homeostasis might be involved in several diseases. Metal ion homeostasis is maintained through highly regulated processes of uptake, storage, and secretion, and a specific set of transporters is active in each cellular compartment to provide a delicate balance of transport activities across their membranes [91–93].

At present, only iron metabolism has been investigated in patient with migraine. Iron homeostasis is essential for normal metabolic and neurological function. Several neuroimaging studies investigated iron deposition in pain-regulatory nuclei in the brain of both episodic and chronic migraine patients and suggested that iron deposition in periaqueductal gray matter may be a marker of chronic migraine [94]. In addition, patients with iron-deficiency anemia have a high frequency of migraine attacks and an association between body iron storage status and the incidence of migraine, especially among females, has been suggested. However, further studies are required to provide additional experimental and clinical data.

Finally, the potential role in pathogenesis as well as therapy of additional metal ion, like, zinc, copper, and lead, is still under investigation. Increased plasma concentrations of lead and copper were observed in some studies but were unchanged in others.

9. Glucose metabolism

In 2001, an association between five polymorphisms of the insulin receptor gene (*INSR*) and migraine was described, supporting clinical evidences suggesting a close relationship between impaired cerebral glucose metabolism and migraine [95,96]. At present, no mutations in *INSR* gene have been found in migraineurs. However, recent 18-fluorodeoxyglucose positron emission tomography (FDG-PET) studies showed several areas of altered glucose metabolism both in MA and MO [97].

The *INSR* gene is located on chromosome 19p13.2 and encodes the insulin receptor protein, a member of the ligand-activated receptor and tyrosine kinase family of transmembrane signaling proteins. The protein is proteolytically processed to generate alpha and beta subunits that form a heterotetrameric receptor. The insulin receptor is activated also by insulin growth factor I and II, and receptor binding induces auto-phosphorylation of the beta subunit at various tyrosine residues. The final effect of insulin receptor stimulation includes translocation of the glucose transporter proteins (GLUT1, GLUT4), promoting glucose influx in different cells. In addition, insulin receptors regulate several complex physiological actions like the synthesis and storage of carbohydrates, lipids, and proteins. Insulin receptors are abundantly distributed throughout the brain, and insulin action produces several behavioral and metabolic effects within the CNS [98].

The *SLC2A1* gene encodes the Glucose transporter 1 (GLUT1) protein. GLUT1 deficiency syndrome (GLUT1DS) is a well-described metabolic disorder that results from impaired glucose transport into the CNS [99]. The syndrome classically presents with infantile-onset epilepsy, progressive microcephaly, and developmental delay. However, in a minority of patients, GLUT1DS may cause mainly hemiplegic migraines.

The precise mechanisms of glucose metabolism impairment in migraine need to be further elucidated. However, the modulation of this metabolic pathway in migraine is of particular interest. Several dietary regimens have been suggested for migraine prevention and the ketogenic diet, a diet that leads to the elevation of ketone bodies, has recently shown great promise in the prevention of migraines.

10. Conclusions

In the last two decades, studies in experimental animals and humans have provided a significant advance in the understanding of migraine pathophysiology. Genetic studies, both in patients with familial and sporadic migraine, provided several intriguing suggestions that contributed to elucidate the complex molecular mechanisms underlying the disease.

In this review, we discussed recent data provided by genetic studies in migraine, suggesting common molecular pathways that could be investigated as new therapeutic targets. Involvement of circadian clocks, ion channels, synaptic plasticity, vascular factors, ion metal homeostasis, and impaired glucose metabolism has been demonstrated in the pathophysiology of the disease, and a role for these metabolic pathways as potential targets for migraine prophylaxis has been suggested. However, there is an urgent need to merge into a common molecular pathway the different abnormalities discovered in order to find a common pathogenetic link between different genetic and metabolic abnormalities observed so far. Additional investigations are needed in order to reach this goal.

11. Expert opinion

Migraine is a complex disease, manifesting itself with a great variability in the symptoms of the acute attacks as well in the characteristics of the interictal periods. Therefore, the treatment response to both acute and prophylactic therapies shows a great variability. At present, patient's satisfaction with prophylactic migraine therapy is generally reported to be low. Lack of efficacy of prescribed medications is reported by more than 50% of migraineurs. In addition, approximately 50% of patients reported bothersome side effects and often halt prophylactic therapy. The recent development of monoclonal antibodies against calcitonin gene-related peptide (CGRP) or one of its receptors (RAMP1), the first drugs specifically designed to act on one of the pathophysiological mechanisms of the disease, offered considerable improvements over existing drugs. However, approximately one-third of migraine patients do not respond to these drugs and the long-term effects of such treatment are still unknown. Therefore, there is still substantial clinical interest in developing new drugs acting on the complex biological mechanisms of the disease.

Molecular genetic studies are trying to disentangle the disease complexity, showing that migraine is located at the crossroads of several metabolic pathways. In this review, we have discussed the more recent data concerning the pathways

that, according to molecular genetic studies, are involved in migraine pathogenesis. Several studies suggested that protein involved in the regulation of circadian rhythms, ion-channel function, synaptic plasticity, vascular factors, ion metal homeostasis, and glucose metabolism may have a role in migraine. However, these pathways are very complex and are still necessary to find a common molecular mechanism. Some studies are trying to integrate these different pathways. For example, one recent study in rats found that cerebral glucose metabolism is an important modulator of CSD, the pathophysiological mechanism of the migraine aura. Hyperglycemia renders the cortex more resistant to CSD initiation and hastens CSD recovery, whereas hypoglycemia had the opposite effect on CSD durations [100]. In addition, several genes associated with migraine phenotype in GWAS, like *REST, LP1,* and *NRP1*, have a role in different molecular mechanisms previously described like vascular function, and metal ion homeostasis. More basic studies are necessary to elucidate the potential interaction of these different mechanisms.

At present, based on available scientific data, only two novel therapeutic strategies are promising in migraine. The first one is related to the prominent role of the hypothalamus in the disease and to the function of PACAP38 and its receptors (i.e., PAC1, VPAC1, VPAC2) in regulating circadian clocks. In addition, the peptide is a well-known vasoactive molecule and modulated production of inflammatory molecules. Monoclonal-humanized antibodies against PACAP38 have been developed and one of these (AMG 301) is already under investigation in a Phase II clinical trial. Data on safety and efficacy of such treatment are expected. Further experimental and clinical research in this field is very much warranted.

The second one is related to the 'new' channels that are under investigation in disease pathogenesis. Transient receptor potential (TRP) channels have been repeatedly linked to migraine and one of these, TRPM8 channel, is an interesting target for the disease therapy. The channel is activated by pathological stimuli related to headache attacks, like cold, and may be modulated by several drugs. Intriguingly, some studies indicated that TRPM8 blockade is necessary to reduce pain but also channel activation induces analgesia. Additional basic studies are warranted to better elucidate this complex phenomenon that may be of relevance in the process of migraine chronification. Agonist (like IGM-5) and antagonist (IGM-18) of the TRPM8 channel have been discovered and are currently under investigation in order to evaluate their ability to modulate both acute and chronic pain.

In conclusion, a significant advance in our understanding of migraine molecular pathways was observed in recent years and the possibility of developing new strategies for migraine prevention using a genetic approach to disease therapy is becoming increasingly real.

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