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Migraine: disease characterisation, biomarkers, and precision medicine

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Migraine is a disabling neurological disorder, diagnosis of which is based on clinical criteria. A shortcoming of these criteria is that they do not fully capture the heterogeneity of migraine, including the underlying genetic and neurobiological factors. This complexity has generated momentum for biomarker research to improve disease characterisation and identify novel drug targets. In this Series paper, we present the progress that has been made in the search for biomarkers of migraine within genetics, provocation modelling, biochemistry, and neuroimaging research. Additionally, we outline challenges and future directions for each biomarker modality. We also discuss the advances made in combining and integrating data from multiple biomarker modalities. These efforts contribute to developing precision medicine that can be applied to future patients with migraine.

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This is the second in a **Series** of three papers about migraine

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Introduction

Migraine is a highly prevalent neurological disorder, listed as the second leading cause of years lived with disability worldwide.[1] The pathogenesis of migraine has a strong genetic component and involves activation of trigeminovascular pain pathways.[2–5] Migraine is defined solely by clinical criteria, which has fuelled research efforts to establish migraine-specific biomarkers for precision medicine approaches.[5,6] Advances in genetics, provocation models, biochemistry, and neuroimaging hold great promise and have improved our understanding of migraine pathogenesis. In this Series paper, we first evaluate the progress that has been made in the search for migraine-specific biomarkers. Second, we discuss the use of integrating data from multiple biomarker modalities to more accurately assess distinct and unique features of migraine. Finally, we highlight challenges with the current biomarker approaches and provide recommendations to improve research into biomarkers of migraine.

Classification and characterisation of migraine

The diagnosis of migraine is based on clinical criteria provided in the third edition of the International Classification of Headache Disorders (ICHD-3).[6] Medical history is the main component of diagnosis and typical clinical features include recurrent headache attacks of unilateral location, pulsating quality, moderate or severe intensity, aggravation by routine physical activity, and association with nausea, vomiting, photophobia, and phonophobia.[6] Although migraine pain often lateralises to one side of the head, approximately 40% of patients report migraine pain of bilateral location.[7] However, migraine is a heterogeneous disorder with multiple subphenotypes;[6] therefore, the ICHD-3 has defined clinical criteria for migraine without aura, migraine with aura, and rarer subphenotypes (panel 1).[6] Individuals with migraine most often have a normal physical examination, with no findings suggestive of another underlying cause for headache. Neuroimaging is therefore rarely needed in the diagnostic investigations.[8]

Aura occurs in approximately one-third of individuals with migraine and is characterised by transient focal neurological symptoms of recurrent nature that develop gradually over 5–60 min.[6] Visual symptoms (eg, scotoma or fortification spectra) are the most frequent clinical manifestation of aura, occurring in more than 90% of individuals with migraine with aura.[6] Less common are sensory symptoms (ie, paraesthesia) and speech or language disturbances, both of which are usually present in conjunction with visual aura symptoms.[6] Although the aura phase typically occurs before the onset of headache, some data suggest that aura symptoms are relatively frequent during or in the absence of headache as well.[9] Another important aspect of migraine classification is the diagnosis of chronic migraine.[6] The ICHD-3 defines chronic migraine as headache occurring on 15 or more days per month, of which at least 8 days fulfil the clinical criteria for migraine with or without aura.[6]

Search strategy and selection criteria

We searched MEDLINE (from database inception to Jan 1, 2020), and Embase (from database inception to Jan 1, 2020). We used the search terms "migraine" in combination with the terms "diagnosis", "classification", "genetic", "provocation", "human models", "blood biomarkers", "serum", "brain", "cortical changes", "imaging", "data integration", "biomarker", "CGRP", "PACAP" and/or "signaling molecule". We mainly selected publications from the past 5 years but did not exclude other publications that were commonly cited and highly regarded. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. No language restrictions were used in the search strategy.

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Panel 1: **ICHD-3 diagnostic criteria for migraine**

Migraine without aura
- Criterion A: at least five attacks fulfilling criteria B–D
- Criterion B: headache attacks lasting 4–72 h (when untreated or unsuccessfully treated)
- Criterion C: headache has at least two of the following four characteristics:
  - Unilateral location
  - Pulsating quality
  - Moderate or severe pain intensity
  - Aggravation by or causing avoidance of routine physical activity (eg, walking or climbing stairs)
- Criterion D: during headache, at least one of the following:
  - Nausea, vomiting, or both
  - Photophobia and phonophobia
- Criterion E: not better accounted for by another ICHD-3 diagnosis

Migraine with aura
- Criterion A: at least two attacks fulfilling criteria B and C
- Criterion B: one or more of the following fully reversible aura symptoms:
  - Visual
  - Sensory
  - Speech or language
  - Motor
  - Brainstem
  - Retinal
- Criterion C: at least three of the following six characteristics:
  - At least one aura symptom spreads gradually over ≥5 min
  - Two or more aura symptoms occur in succession
  - Each individual aura symptom lasts 5–60 min
  - At least one aura symptom is unilateral
  - At least one aura symptom is positive
  - The aura is accompanied, or followed within 60 min, by headache
- Criterion D: not better accounted for by another ICHD-3 diagnosis

Chronic migraine
- Criterion A: headache (migraine or tension type) on ≥15 days per month for >3 months, and fulfilling criteria B and C
- Criterion B: occurring in a patient who has had at least five attacks fulfilling criteria B–D for migraine without aura, or criteria B and C for migraine with aura
- Criterion C: on ≥8 days per month for >3 months, fulfilling any of the following:
  - Criteria C and D for migraine without aura
  - Criteria B and C for migraine with aura
  - Believed by the patient to be migraine at onset and relieved by a triptan or ergot derivative
- Criterion D: not better accounted for by another ICHD-3 diagnosis

Probable migraine
- Criterion A: attacks fulfilling all but one of criteria A–D for migraine without aura, or all but one of criteria A–C for migraine with aura
- Criterion B: not fulfilling ICHD-3 criteria for any other headache disorder
- Criterion C: not better accounted for by another ICHD-3 diagnosis

ICHD-3=International Classification of Headache Disorders, third edition.

As migraine is increasingly being recognised as a heterogeneous disorder, the ICHD-3 has provided clinical criteria for probable migraine, which allows for a diagnosis pending confirmation during the early clinical evaluation.[6]

Genetic biomarkers

Migraine often clusters in families, suggesting a strong genetic component to its pathogenesis.[2] However, identifying the relevant genes remains a challenge. Population-based twin and family studies have shown that migraine is a complex neurological disorder, with its features probably arising from gene–gene and gene–environment interactions, but also other unknown factors.[2,10] A genome-wide association meta-analysis identified 38 genomic loci that affect migraine risk.[10] This meta-analysis also found an enrichment of migraine risk variants in genes expressed in tissues with vascular and smooth muscle cell components. This finding is consistent with previous reports of a shared genetic basis of migraine with ischaemic stroke and coronary heart disease.[11,12] A summary analysis confirmed the presence of cardiovascular enrichment in individuals with migraine, although analysis of chromatin data yielded evidence in support of neuronal enrichment as well.[13] Based on these findings, future multiple-tissue analyses should emphasise representation of the highest possible number of tissues and cell types.

Genetic studies have also provided mechanistic insights to improve understanding of migraine subphenotypes.[14] In an analysis of 1589 families with migraine, high polygenic load was associated with increased migraine severity, earlier age of onset, and migraine with aura.[15] Of note, family history of migraine alone might suffice to make similar estimations, since increased prevalence of migraine in the family has been associated with an earlier age of onset, migraine with aura, and an increased number of medication days.[16]

Evaluation of the epigenetic contribution in migraine pathogenesis is also important. A genome-wide association analysis has quantified patterns of DNA methylation in migraine and found 62 independent differentially methylated regions.[17] However, this study did not distinguish between migraine with and without aura.

Further studies are needed because research into epigenetic contributions in migraine is still in its infancy.

Stratification by genetics has offered biomarker advancement in migraine studies and enabled identification of rare monogenic disorders related to migraine with aura (panel 2).[2,9,18] These disorders include familial hemiplegic migraine, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations, and familial advanced sleep phase syndrome.[14] Additionally, concerted efforts to delineate additional genes as biomarkers for other rare monogenic subtypes of migraine are ongoing.[14] However, it remains difficult to identify causal genes in common polygenic subtypes and to define the mechanisms that increase the risk of migraine.

Challenges and future perspectives

The combination of multiple genetic variants with small effect sizes and environmental factors has hampered mapping of genetic biomarkers for common subtypes of migraine. Therefore, future studies are likely to focus on examining the association between clinical features and possible genetic biomarkers. Additionally, identification of genetic risk factors might contribute to developing precision medicine approaches to individualise treatment strategies. One proof-of-concept study, published in 2019, found an association between high polygenic load and improved response to triptans in individuals with migraine.[19] This study is the first step in genetics-guided treatment strategies for migraine in the era of precision medicine. Large-scale prospective studies are needed to further explore the potential of pharmacogenetics.

Progress made in genetic investigations have enabled the identification of approximately 40 loci that independently contribute to the biological underpinnings of migraine.[10] The genetic contribution is, therefore, polygenic for common types of migraine, with each identified risk variant likely to account for only modest effects. However, these risk variants offer novel insights that should improve our understanding of signalling pathways underlying migraine pathogenesis and enable identification of mechanism-based drug targets.

Mendelian randomisation is a novel approach, in which genetic variants are used to determine whether an observational association between a risk factor and an outcome is consistent with a causal effect.[20] The benefits of mendelian randomisation include a theoretical random distribution of genetic variants as genotypes are passed on randomly through meiosis. Although mendelian randomisation holds great promise, it requires a robust association of a genetic variant to the risk factor. Additionally, mendelian randomisation relies on the assumptions that the genetic variant does not affect the outcome through a mechanism independent of the risk factor in question and does not influence independent factors that confound the risk factor–outcome relationship. Thus, data should be interpreted with caution as mendelian randomisation emerges in migraine studies.

Panel 2: **Genetics in migraine**

Key facts

- Based on studies with twins, the heritability of migraine has been estimated as 42%[2]
- A genome-wide association meta-analysis identified 38 genomic loci that affect migraine risk[9]
- The relative risk of migraine without aura is 1·9 in first-degree relatives of probands with migraine without aura[18]
- The relative risk of migraine with aura is 3·8 in first-degree relatives of probands with migraine with aura[18]

Genetic biomarkers for monogenic subtypes of migraine or migraine-related syndromes

- Familial hemiplegic migraine
  - Type 1 (*CACNA1A* gene)
  - Type 2 (*ATP1A2* gene)
  - Type 3 (*SCN1A* gene)
- Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (*NOTCH3* gene)
- Retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (*TREX1* gene)
- Familial advanced sleep phase syndrome (*CSNK1D* gene)

Provocation biomarkers

The pathogenesis of migraine is multifaceted, with a complex interplay between different molecular signalling pathways.[5] A key feature of migraine is that various trigger factors have been shown to produce migraine attacks (figure 1).[21] This feature provides a unique opportunity to identify signalling pathways that cause migraine through human provocation models, wherein endogenous signalling molecules or other putative triggers are used to induce migraine in humans.[21] An important observation from human provocation studies is that only individuals with migraine develop provoked migraine attacks, whereas healthy volunteers develop, at most, a mild headache.[21]

In principle, human provocation models apply a double-blind, crossover design whereby individuals with migraine or healthy volunteers are randomly allocated to receive a putative trigger molecule or placebo.[21] A headache diary is used to record headache occurrence, characteristics, and accompanying symptoms.[21] Notably, provoked migraine attacks must fulfil at least one of two categories. Category one describes headache with at least two of the following clinical features: unilateral location, pulsating quality, moderate to severe pain intensity, and aggravation or avoidance of routine physical activity. Additionally, headache must be accompanied by at least one of the following symptoms: nausea, vomiting, or

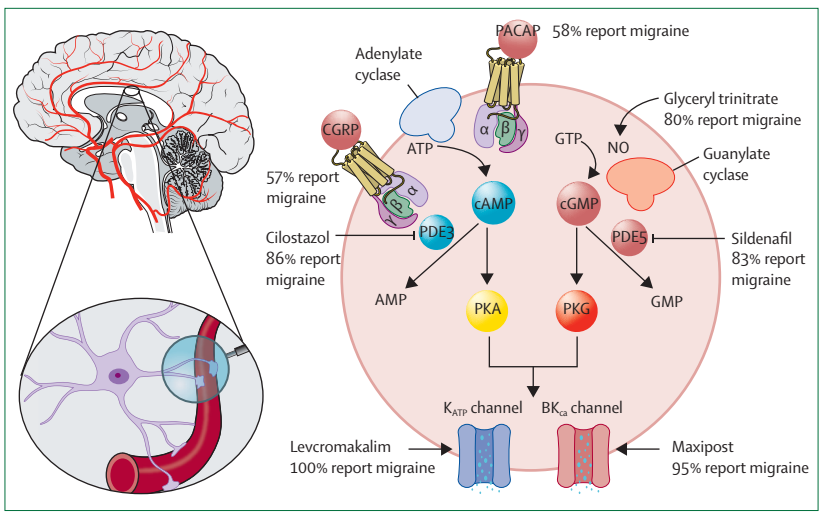

Figure 1: **Molecular signalling pathways in migraine**
Cell is a vascular smooth muscle cell. Molecular signalling pathways underlying migraine pathogenesis have been studied using provocation models, in which putative trigger molecules are used to induce migraine attacks in humans. These trigger molecules include CGRP, PACAP, glyceryl trinitrate, cilostazol (PDE3 inhibitor), sildenafil (PDE5 inhibitor), and levcromakalim (K_{ATP} channel opener). cAMP=cyclic adenosine monophosphate. cGMP=cyclic guanosine monophosphate. CGRP=calcitonin gene-related peptide. K_{ATP}=adenosine triphosphate-sensitive potassium. PACAP=pituitary adenylate cyclase-activating polypeptide. PDE3=phosphodiesterase 3. PDE5=phosphodiesterase 5. PKA=protein kinase A. PKG=protein kinase G.

photophobia and phonophobia. Category two describes headache that mimics the patient's usual migraine attacks and is treated with a rescue medication.

In 1993, the first migraine provocation study showed that individuals with migraine develop more severe headache than healthy volunteers following intravenous administration of the nitric oxide donor, glyceryl trinitrate.[22] Since then, various putative trigger molecules have been tested for their ability to induce migraine, including calcitonin gene-related peptide (CGRP), pituitary adenylate cyclase-activating polypeptide (PACAP), an adenosine triphosphate-sensitive potassium (K_{ATP}) channel opener, and a large conductance calcium-activated potassium (BK_{Ca}) channel opener.[5,21,23]

Intravenous infusion of CGRP or PACAP induces migraine attacks in approximately 60% of individuals with migraine.[24,25] Higher induction rates (≥80%) have been observed following administration of glyceryl trinitrate and phosphodiesterase 3 and 5 inhibitors.[22,26,27] A common factor for all trigger molecules is that they mediate their intracellular effects through the second messenger systems of either cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP).[21] Based on these findings, it was hypothesised that downstream effects of cAMP and cGMP signalling could involve modulation of ion channels, mainly potassium channels.[22] It was subsequently shown that administration of a K_{ATP} channel opener yielded a migraine induction rate of 100% in individuals with migraine, whereas the corresponding induction rate was 95% following administration of a BK_{Ca} channel opener (figure 1).[23,28] Administration of a K_{ATP} channel opener was also found to induce migraine aura in 10 (59%) of 17 patients with migraine with aura.[29] A fundamental question raised by these provocation studies pertains to the site of action, with some proponents in favour of a peripheral origin of migraine, whereas others have argued that a central origin is more probable.[4,21]

Challenges and future perspectives

Human provocation models have provided insight into signalling pathways underlying migraine pathogenesis. These studies have also contributed to the identification and development of drugs that target specific trigger molecules. This contribution is most evident with the recently (2018–20) approved drugs targeting CGRP or its receptor, which have proven effective in acute and preventive treatment of migraine.[30] Consequently, future drug development should, in part, be guided by discoveries from human provocation studies. From this perspective, two potential drug targets are K_{ATP} channel blockers and BK_{Ca} channel blockers, as opening these channels provoked migraine attacks in nearly all study participants with migraine.[23,28] However, there are also limitations to human provocation models.[21] For example, selective inhibition of nitric oxide synthase (NOS) has been suggested as a possible drug target for migraine based on two key facts: glyceryl trinitrate induces migraine attacks and administration of a non-selective NOS inhibitor led to headache relief in individuals with migraine.[22,31] However, inducible nitric oxide inhibition did not abort or prevent migraine attacks.[32,33]

Apart from discovery of drug targets for migraine, human provocation models could also be used as a biomarker to predict efficacy of mechanism-based therapies, such as blockers of CGRP signalling.[21] Large-scale registry studies are needed, in which individuals with migraine are initially provoked by intravenous infusion of CGRP and subsequently allocated to receive treatment with a blocker of CGRP signalling, such as monoclonal antibodies against CGRP or its receptor. The hypothesis is that individuals with migraine who develop provoked migraine attacks following CGRP infusion would benefit more from treatment with such drugs than would those who did not develop provoked attacks after CGRP infusion. This rationale remains speculative and rigorous investigations are needed to ascertain whether human provocation models can be used to predict the treatment response in individuals with migraine.

Blood biomarkers

Research into blood biomarkers of migraine has garnered attention over the past 10 years.[4] This interest is fuelled by the concept that blood biomarkers contribute to the understanding of molecular mechanisms underlying migraine. Efforts have been made to establish blood biomarkers that could predict and monitor treatment response in individual patients. Blood biomarker studies have investigated a multitude of circulating signalling

molecules implicated in migraine pathogenesis.[4] Herein, we focus our discussion only on blood biomarker studies of CGRP and PACAP.

Ictal phase

In 1990, the first study investigated plasma concentrations of CGRP in the external jugular vein during spontaneous migraine attacks.[34] This study showed that CGRP plasma concentrations were elevated in individuals with migraine, compared with a control population. Subsequently, another study reported that ictal (ie, during migraine attacks) plasma concentrations of CGRP were also elevated in peripheral blood.[35] However, these findings were not reproduced in a study that assessed CGRP plasma concentrations in both the external jugular vein and peripheral blood, using two different assays.[36] Regarding ictal changes of PACAP, two studies have reported elevated PACAP-like immunoreactivity during spontaneous migraine attacks.[37,38]

Interictal phase

Measurements of blood biomarkers have been done in the interictal phase (ie, between migraine attacks) in individuals with both episodic and chronic migraine. The available data are highly conflicting, with strikingly different findings. Two studies have reported elevated interictal plasma concentrations of CGRP in individuals with both episodic and chronic migraine, compared with healthy participants.[39,40] However, these findings were not reproduced by another study that found no differences in serum CGRP concentrations between individuals with chronic migraine, those with episodic migraine, and healthy participants.[41] In terms of PACAP, two studies have found no increases in the interictal phase of migraine.[38,42]

Prediction of treatment response

Two studies have reported higher baseline concentrations of CGRP in individuals with migraine who subsequently benefited from preventive treatment with onabotulinumtoxinA, compared with those who did not report therapeutic benefit.[43,44] However, this finding was not reproduced in another study.[41] Therefore, it remains unknown whether blood biomarker measurements can reliably predict treatment response in individuals with migraine.

Challenges and future perspectives

Research into blood biomarkers of migraine is still in its infancy, with much work left to be done. The discordant findings might be explained by methodological limitations, small sample sizes, and differences in study design and assays. Therefore, further investigations are needed to optimise data accuracy and reproducibility. An absence of standardised methods for data collection and sample processing hampers comparisons between studies. For example, assays used in CGRP studies are extremely variable and not well validated. Suboptimal assay validation leads to an inability to confidently determine whether the assay only detects the blood biomarker of interest. For example, ELISA assays are used to detect CGRP and PACAP, but these assays could also detect close relatives, such as PACAP-38 versus PACAP-27 or αCGRP versus βCGRP versus amylin (~40% identical sequence to CGRP).[45] Most assays (ie, radioimmunoassay or ELISA) use antibodies to detect peptides but antibodies can often detect both peptide fragments and the intact peptide. Hence, it is important to describe results as CGRP-like and PACAP-like immunoreactivity. Each assay must initially be validated through a rigorous process that accounts for sensitivity, specificity, interassay and intra-assay variability, and the effect of matrix interference (ie, serum or plasma). There are many aspects of sample processing that can affect results, such as use of plasma or serum, time delays, presence of protease inhibitors (which can interfere in assays), composition of storage tubes, and freeze–thaw cycles. All samples must also fall within the linear range of the assay. Researchers should follow appropriate guidance documents (eg, Bioanalytical Method Validation by the US Food and Drug Administration) and adequately report their methods. Commercially available assays infrequently have sufficient validation to give confidence in the results. Aside from assay validation, there is also a need for studies with large samples and appropriate control groups. Future studies should consider a shift from single-biomarker approaches to a panel of multiple biomarkers. Such an approach might show improved separation between groups and yield reproducible data that are needed for validation of blood-based biomarkers for migraine.

For the **US Food and Drug Administration Bioanalytical Method Validation** see https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf

Imaging biomarkers

In studies on biomarkers for migraine, MRI has emerged as useful technology to identify structural and functional changes in individuals with migraine. Alterations in functional connectivity have been investigated in both the interictal and ictal phase of a migraine attack.[46]

Structural imaging

Numerous MRI studies have examined differences in brain structure of individuals with migraine versus healthy controls and differences between migraine with and without aura. White matter hyperintensities have been extensively studied, but with conflicting results.[47] Notably, a meta-analysis, published in 2013, of population-based studies found an association of white matter hyperintensities with migraine with aura, but not migraine without aura, when compared with controls.[47] Additionally, when directly comparing migraine with aura versus migraine without aura, there was no difference in terms of white matter hyperintensities.[47] A population-based MRI study, published in 2016, found no association between white matter hyperintensities and migraine with aura.[48]

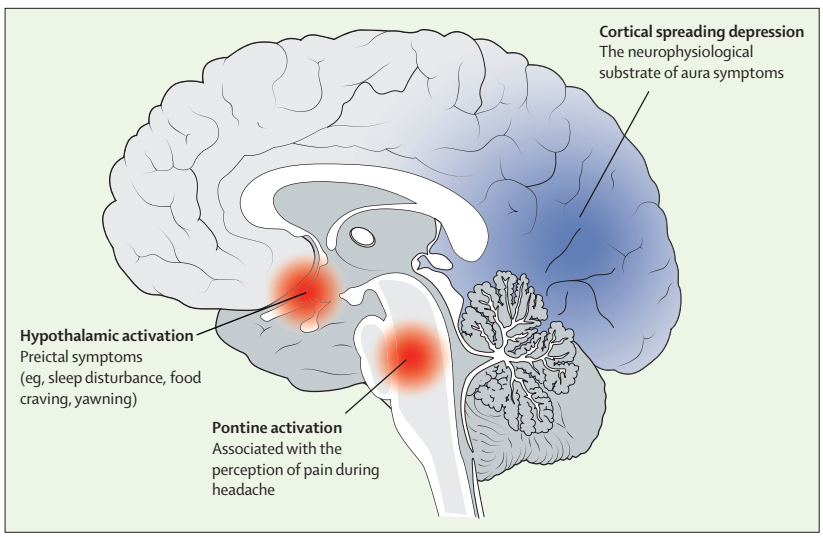

Figure 2: **Functional cerebral changes in the migraine brain**
The preictal phase of migraine has been linked to hypothalamic activation, whereas the headache phase of migraine has been associated with increased activity within the dorsal pons. Regarding the aura phase of migraine, imaging studies have shown blood flow changes consistent with cortical spreading depression. Several imaging studies have also reported interictal cerebral changes in various functional networks (not shown in the figure).

Other MRI studies have assessed differences in cortical parameters (eg, thickness, volume, surface area) and white matter tract integrity. For instance, one population-based MRI study assessed differences in cortical thickness in women with migraine with aura compared with a control population of women without migraine and found thicker cortex corresponding to visual areas in the migraine group.[49] Additionally, one diffusion-tensor imaging study found structural changes in ascending (ie, trigeminothalamic tract or thalamocortical tract) and descending pain pathways (ie, periaqueductal grey), which could correspond to thalamic changes reported from one multicentre study.[50,51] Findings from these and other MRI studies are important since structural imaging might be used to support or supplement migraine diagnosis in patients who are difficult to classify on the basis of their clinical symptoms alone. This topic was explored in a proof-of-concept study,[52] in which the authors used cortical classifiers (ie, thickness, volume, or surface area) to determine whether an individual had episodic migraine, chronic migraine, or was a healthy control. The cortical classifiers were fairly accurate for chronic migraine versus healthy controls, with an 86·3% classifier accuracy. Larger samples (by comparison with existing studies) and new advents in imaging technologies will undoubtedly continue to explore the potential and feasibility of structural imaging-based diagnostic classification models. Notably, some studies have found differences in brain structure when comparing individuals with migraine to those with other headache disorders, such as tension-type headache and headache attributed to traumatic brain injury.[53,54]

Functional Imaging

Functional MRI (fMRI) studies have shown that migraine is associated with changes in functional connectivity and stimulus-induced activation of cerebral circuits related to pain processing areas and visual systems.[46,55–59] Additionally, a combined positron emission tomography and MRI study, published in 2019, showed glial activation in pain processing areas of migraine with aura patients, compared with healthy controls.[60] An increasing number of fMRI studies have also begun to assess the use of interictal functional connectivity data to develop imaging biomarkers for various purposes, such as diagnostic classification of migraine and prediction of migraine attack frequency.[61–64] Similar to structural imaging, these functional imaging biomarker models require further refinement and validation in multicentre settings.

The preictal phase starts up to 48 h before onset of migraine headache. It manifests with clinical features (eg, sleep disturbances, food craving) that have been linked to hypothalamic activation in both spontaneous and glyceryl trinitrate-induced migraine attacks, as measured by fMRI.[65,66] Regarding the aura phase of migraine, imaging studies have shown functional changes consistent with cortical spreading depression, which is widely believed to be the underlying neurophysiological cause of substrate.[4,67,68]

Functional imaging studies have shown increased activity within the dorsal pons during the headache phase of spontaneous migraine attacks.[69,70] This finding was also reproduced following glyceryl trinitrate-induced migraine attacks, in which activation of the dorsal pons was ipsilateral in those with unilateral migraine attacks and bilateral in those with bilateral migraine attacks (figure 2).[71] Collectively, these data have led to the conclusion that dorsal pontine activation is probably an imaging biomarker of the headache phase of migraine. Notably, increased functional connectivity has also been shown between the pons and somatosensory cortex during attacks in migraine with aura.[72]

Challenges and future perspectives

Structural and functional imaging studies have provided key insights into migraine pathogenesis and set the stage for development of imaging-based biomarkers. Future research should focus on refining imaging biomarkers, improving their accuracy, determining their sensitivity and specificity, and ultimately validating them for clinical use. For this purpose, standardised imaging protocols should be implemented to ensure high-quality data acquisition and enable comparative assessments. To provide additional pathophysiological insights, large-scale imaging studies are needed to assess structural and functional differences among different headache disorders with overlapping clinical features. Additionally, future imaging studies should also investigate whether imaging techniques can be used to predict treatment responses. An important consideration for fMRI studies

should involve the use of data-driven analyses and validation of results by an independent research group. This consideration would enable more robust findings and increase the likelihood of reproducibility. Awaiting such imaging studies, we must continue to encourage more innovative approaches to delineate imaging biomarkers of migraine.

Integration of biomarker modalities

Integration of biomarker modalities offers a promising way to combine data from multiple sources and identify novel biomarkers for migraine. Additionally, such approaches might advance our understanding of disease mechanisms underlying migraine and several studies have sought to combine biomarker modalities and establish strategic interdisciplinary research collaborations. We herein summarise the results from studies that have used at least two of the four biomarker modalities.

A combination of genetic and provocation biomarker modalities has been used to investigate the effects of CGRP in individuals with familial hemiplegic migraine. CGRP did not induce migraine attacks in patients with familial hemiplegic migraine who had known ion channel mutations and in those who did not.[73,74] This conclusion contrasts with the findings reported in common types of migraine.[21] Additionally, another provocation study found no association between high family load (≥2 first-degree relatives with migraine) and migraine induction rate following PACAP infusion in individuals with migraine without aura.[75]

Another combination of modalities includes neuroimaging and human provocation models. Three studies have used magnetic resonance angiography (MRA) to record vascular changes following provoked migraine attacks in individuals with migraine without aura. The first MRA study found that CGRP-induced migraine attacks were accompanied by dilation of both the middle cerebral artery (MCA) and middle meningeal artery (MMA).[76] MCA and MMA dilation were only present on the pain side in those who developed unilateral migraine attacks.[76] In another MRA study, MCA and MMA changes were recorded after migraine induction using a phosphodiesterase 3 inhibitor.[77] The authors reported that the provoked attacks were associated with an MMA dilation on the pain side, but no dilation of the MCA. Additionally, one MRA study found that PACAP-induced migraine attacks were associated with MMA dilation but not MCA dilation;[78] the authors found no association between provoked attacks and pain location.

Neuroimaging and provocation models have also been combined to examine changes in functional connectivity before and at onset of provoked migraine attacks. In one randomised, double-blind resting state fMRI study, abnormal functional connectivity was found in all investigated cerebral networks (sensorimotor, salience, and default mode) following intravenous infusion of PACAP.[79] No changes in functional connectivity were found after intravenous infusion of vasoactive intestinal peptide (active placebo). All of the investigated cerebral networks had previously been implicated in processing of nociception and emotions.[80,81]

Conclusions

Biomarker research has already made great contributions to our understanding of migraine pathogenesis. Advancements in genetics, provocation models, biochemistry, and neuroimaging have shown the potential of biomarker-driven approaches to diagnosis, treatment, and drug discovery. Efforts to combine biomarker modalities have improved understanding of the biological complexity underlying migraine and its subtypes. Building on this foundation, future research should investigate precision medicine approaches that improve the diagnosis and treatment of migraine.

Contributors

MA, HA, MA-MA-K, and PJG initiated the concept and designed the scope of this Series paper. All authors contributed to the review of the relevant literature and to the writing of the Review. All authors reviewed and approved the final version.

Declaration of interests

MA is a consultant, speaker or scientific advisor for AbbVie, Allergan, Amgen, Alder, Biohaven, Eli Lilly, Lundbeck, Novartis, and Teva, and primary investigator for Alder, Amgen, Allergan, Eli Lilly, Lundbeck, Novartis and Teva trials. MA has no ownership interest and does not own stocks of any pharmaceutical company. MA serves as associate editor of *Cephalalgia*, and associate editor of the *Journal of Headache and Pain*. MA is president of the International Headache Society. GMT reports grants or consultancy support from Novartis, Lilly, Teva, and Allergan, and independent support from the Dutch Research Council, National Institutes of Health, European Community, Dutch Heart Foundation, and Dutch Brain Foundation. MA-MA-K has acted as an invited speaker for Novartis and received a travel grant from ElectroCore. MJL reports grants from the National Research Foundation of Korea, Korean Society of Neurosonology, and Yuhan Company. MJL is also a consultant, speaker or scientific advisor for Eli Lilly and has received speaker honoraria from Sanofi-Aventis and YuYu Pharma, outside the submitted work. MJL serves as junior editor of *Cephalalgia*. DLH reports grants from the Royal Society of New Zealand (Marsden Fund), Living Cell Technologies, and the Maurice Wilkins Centre, during the conduct of the study. DLH is a consultant, speaker or scientific advisor for Eli Lilly, Intarcia Therapeutics, Merck Sharp & Dohme, and Amgen–Novartis, outside the submitted work. DLH serves as an editor of the *British Journal of Pharmacology*. LHS has received speaker honoraria from Allergan, outside the submitted work. AJS reports grants from Sir Jules Thorn Award for Biomedical Research, during the conduct of the study. AJS reports personal and speaker fees for a single lecture in 2019 from Novartis, and received funding for a 12-month clinical headache fellowship at the Department of Neurology, Queen Elizabeth Hospital (Birmingham, UK). AJS is also funded by Allergan and reports personal fees from participation on advisory boards for Novartis and Allergan, outside the submitted work. TJS reports personal fees from Alder, Allergan, Abbvie, Amgen, Biohaven, Cipla, Click Therapeutics, Dr Reddys, Eli Lilly, Equinox, Ipsen, Lundbeck, Novartis, Teva, Weber and Weber, and XoC, research grants from the American Migraine Foundation, Amgen, Henry Jackson Foundation, National Institutes of Health, Patient Centered Outcomes Research Institute, and US Department of Defense. TLS also reports stock options from Aural Analytics and Nocira, and royalties from UpToDate, outside the submitted work. PJG reports, over the last 36 months, grants and personal fees from Amgen and Eli-Lilly, grants from Celgene, and personal fees from Alder Biopharmaceuticals, Aeon Biopharma, Allergan, Biohaven Pharmaceuticals, Clexio, Electrocore, eNeura, Epalex,

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