Migraine

Ashina, Messoud; Terwindt, Gisela; Al-Mahdi Al-Karagholi, Mohammad ; De Boer, Irene; Lee, Mi Ji; Hay, Debbie L; Schulte, Laura H; Hadjikhani, Nouchine; Sinclair, Alex; Ashina, Hakan; Schwedt, Todd; Goadsby, Peter J.

DOI:
10.1016/S0140-6736(20)32162-0

Title: Migraine – Disease Characterization, Biomarkers, and the Path to Precision Medicine.

Authors: Messoud Ashina[1], Gisela M. Terwindt[2], Mohammad Al-Mahdi Al-Karagholi[1], Irene de Boer[2], Mi Ji Lee[3], Debbie L. Hay[4], Laura H. Schulte[5], Nouchine Hadjikhani[6], Alexandra J. Sinclair[7,8], Håkan Ashina[1], Todd J. Schwedt[9], Peter J. Goadsby[10].

Affiliations:

[1]Danish Headache Center, Department of Neurology, Rigshospitalet Glostrup, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

[2]Department of Neurology, Leiden University Medical Center, 2300 WB Leiden, Netherlands.

[3]Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

[4]School of Biological Sciences and Centre for Brain Research, University of Auckland, Auckland, New Zealand.

[5]Department of Systems Neuroscience, University Medical Center Eppendorf, Hamburg, Germany.

[6]A.A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, USA.

[7]Metabolic Neurology, Institute of Metabolism and Systems Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK. a.b.sinclair@bham.ac.uk.

[8]Department of Neurology, University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital, Birmingham, B15 2WB, UK. a.b.sinclair@bham.ac.uk.

[9]Department of Neurology, Mayo Clinic, Phoenix, AZ, USA.

[10]Headache Group, Department of Basic and Clinical Neuroscience, King's College London, London, UK.

Journal: The Lancet

Estimated Word Count: 4,000

Corresponding Author

Professor Messoud Ashina, MD, PhD, DMSc

Danish Headache Center, Department of Neurology
Rigshospitalet Glostrup
Faculty of Health and Medical Sciences, University of Copenhagen
Valdemar Hansen Vej 5, DK-2600 Glostrup, Denmark
Tel: +45 38 63 33 85
E-mail: ashina@dadlnet.dk

Introduction [155 words]

Migraine is a disabling neurological disorder, listed as the 2nd leading cause of years lived with disability worldwide.[1] The pathogenesis of migraine has a strong genetic component[2] and involves activation of different brain regions[3] and the trigeminovascular system.[4] At present, migraine is defined solely on clinical grounds[5], with no validated biomarkers to provide clinicians with actionable information. This has fueled considerable research efforts to establish migraine-specific biomarkers that permit precision medicine approaches. Advances in genetics, provocation models, biochemistry, and neuroimaging provide promise and have greatly improved our understanding of migraine pathobiology. In this Review, we provide an overview of the considerable progress that has been made in the search for migraine-specific biomarkers. Furthermore, we discuss the use of data integration from multiple biomarker modalities as well as big data solutions to more accurately assess distinct and unique features of migraine. Lastly, we highlight challenges with the current biomarker approaches and provide recommendations to improve research into biomarkers of migraine.

Current Approaches to Classification and Characterization of Migraine [414 words]

Migraine diagnosis is based on clinical criteria provided by The International Classification of Headache Disorders- 3rd edition (ICHD-3)[5], with medical history being the mainstay of diagnosis. 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 and/or photophobia and phonophobia.[5] On physical examination, individuals with migraine often appear normal, and neuroimaging is rarely indicated.[6] However, it is important to recognize that diagnosis can be challenging, as migraine can present itself in a multitude of subphenotypes with distinct features.[5,7] As such, the ICHD-3 has defined clinical criteria for *migraine without aura (MO)* and *migraine with aura (MA)* as well as rarer subphenotypes.[5] Migraine aura occurs in about one third of individuals with migraine and is characterized by reversible focal neurological symptoms of recurrent nature that develop gradually over 5 to 60 min.[5] Visual symptoms (e.g. scotoma, fortification spectra) are the most frequent clinical manifestation of aura, occurring in more than 90% of individuals with migraine aura.[8] Less common are sensory symptoms (i.e. paraesthesia) and speech/language disturbances (e.g. aphasia), both of which are usually present in conjunction with visual aura symptoms.[8] It should be noted that the temporal relation between the aura phase and the headache phase of a migraine attack is variable in timing.[8,9] In MA, the aura phase typically occurs before the onset of headache although some data suggests that aura is quite frequent during or in the absence of headache as well.[8,9]

Another important aspect of migraine classification is the diagnosis of chronic migraine.[5] At present, the ICHD-3 defines chronic migraine as headache occurring on ≥ 15 days/month of which at least 8 days fulfil the clinical criteria for MA or MO.[5] Recently, alternative criteria have been proposed and include a recommendation to disregard the need for ≥ 15 headache days/month.[10] This proposed refinement of the current criteria is estimated to double the number of individuals diagnosed with

chronic migraine[10] although further validation is needed in large prospective cohorts before the proposed criteria are implemented in future iterations of the ICHD.

As migraine is increasingly being recognized as a heterogeneous disorder, the International Headache Society has provided clinical criteria for probable migraine to better differentiate individuals with migraine-like attacks from individuals with tension-type headache.[5] It is believed that disease mechanisms underlying probable migraine are similar to those of definite migraine; and that individuals with probable migraine might be more responsive to migraine-specific therapies.

Panel 1. The International Classification of Headache Disorders- 3rd edition (ICHD-3) criteria for migraine without aura, migraine with aura, chronic migraine and probable migraine

Migraine without aura	A. At least five attacks1 fulfilling criteria B–D B. Headache attacks lasting 4–72 hours (when untreated or unsuccessfully treated) C. Headache has at least two of the following four characteristics: 1. unilateral location 2. pulsating quality 3. moderate or severe pain intensity 4. aggravation by or causing avoidance of routine physical activity (e.g. walking or climbing stairs) D. During headache at least one of the following: 1. nausea and/or vomiting 2. photophobia and phonophobia E. Not better accounted for by another ICHD-3 diagnosis.
Migraine with aura	A. At least two attacks fulfilling criteria B and C B. One or more of the following fully reversible aura symptoms: 1. visual 2. sensory 3. speech and/or language 4. motor 5. brainstem 6. retinal C. At least three of the following six characteristics:

	1. at least one aura symptom spreads gradually over ≥5 minutes 2. two or more aura symptoms occur in succession 3. each individual aura symptom lasts 5–60 minutes 4. at least one aura symptom is unilateral 5. at least one aura symptom is positive 6. the aura is accompanied, or followed within 60 minutes, by headache D. Not better accounted for by another ICHD-3 diagnosis.
Chronic migraine	A. Headache (migraine-like or tension-type-like) on ≥15 days/month for >3 months, and fulfilling criteria B and C B. Occurring in a patient who has had at least five attacks fulfilling criteria B–D for migraine with- out aura and/or criteria B and C for migraine with aura C. On ≥8 days/month for >3 months, fulfilling any of the following: 1. criteria C and D for migraine without aura 2. criteria B and C for migraine with aura 3. believed by the patient to be migraine at onset and relieved by a triptan or ergot derivative D. Not better accounted for by another ICHD-3 diagnosis.
Probable migraine	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 B. Not fulfilling ICHD-3 criteria for any other headache disorder C. Not better accounted for by another ICHD-3 diagnosis.

Genetic Biomarkers [860 words]

Migraine often shows a familial aggregation, suggesting a genetic component in migraine pathogenesis.[7,11,12] Identification of genetic risk factors can potentially guide individualized management and improve clinical outcome. Family, twin, and population-based studies reveal that migraine is a genetically complex disorder.[11,13] Complex traits are believed to result from gene-gene and gene-environment interactions, genetic heterogeneity, and potentially other yet unknown reasons. A recent genome wide association meta-analysis[2] identified 38 susceptibility loci that predominantly showed enrichment for genes expressed in vascular and smooth muscle tissues. This is consistent with previously reported shared genetic susceptibility between migraine, stroke and cardiovascular diseases[14,15]. Seven genomic loci were specifically associated with MO, whereas, no loci were associated with MA. This may point towards a higher degree of heterogeneity in the clinical capture.[2] A higher polygenic risk score is associated with migraine severity, a lower-age-at-onset, and migraine with aura.[16] However, based on family history only, an estimation of this can be made as well, where a stronger family history of migraine is associated with migraine with aura, a lower age-at-onset, and a higher number of medication days.[17]

So far, small steps have been taken to dissect the epigenetic contribution towards migraine. The first genome-wide analysis of DNA methylation in migraine identified 62 independent differentially methylated regions in blood samples without distinguishing between migraine without and with aura.[18] Epigenetic mechanisms might be responsible for parts of migraine pathophysiology, for instance in transformation from episodic into chronic migraine with or without acute medication overuse.

Relevant genetic discoveries related to MA or its subtypes derive from investigating monogenetic migraine syndromes, such as Familial Hemiplegic Migraine (FHM1 with *CACNA1A* gene, FHM2 with *ATP1A2* gene, FHM3 with *SCN1A* gene), Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL with *NOTCH3* gene), Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S with

TREX1 gene), and Familial Advanced Sleep Phase Syndrome (FASPS with *CSNK1D* gene).[19] Additional genes have been put forward as possible biomarkers for rare monogenetic migraine subtypes, i.e. *PRRT2*, *SLC1A3*, *SLC4A4,* and *KCNK18,* but evaluation of available data casts doubt on such claims.[19,20] Identification of rare monogenic variants can be more straightforward than for the common polygenic migraine types. But even in these rare conditions more than one gene may play a role, as for instance no major FHM4 gene has been identified despite next generation sequencing efforts.[21] Nonetheless, while many of the traits found in these monogenic subtypes of migraine (e.g. hemiplegia during aura, progressive ataxia) are not found in the common types of migraine, identification of (multiple) causal genes for monogenic subtypes can improve our understanding of the migraine pathogenesis in general, with the potential to facilitate development of new therapeutic approaches.

An interesting future application of genetics in the migraine field will be mendelian randomization (MR).[22] Mendelian randomization uses genetic variants to determine whether an observational association between a risk factor and an outcome is consistent with a causal effect. For example, a genetic variant associated with higher LDL cholesterol levels that also is associated with a higher risk of coronary heart disease would provide supportive evidence for a causal effect of LDL cholesterol on coronary heart disease.[23] MR is an epidemiological study approach that may incorporate genetic information to address questions of causality without being hampered by the typical biases that frequently impact traditional studies, such as confounding and reverse causality (figure 2).[23,24] As genotypes are passed on to offspring during meiosis randomly, this should be unrelated to possible confounding factors. Therefore, MR can be thought of as a 'natural' randomized trial. In a traditional study the association between risk factor (exposure or non-exposure) and outcome is estimated. In a MR study exposure is defined on the presence or absence of a 'risk' allele. It is a prerequisite that the association between this risk allele and exposure should be robust. MR is an upcoming study design with great potential, however, when study assumptions are violated results can be misleading. In the migraine genetics-biomarker field, mendelian randomization is still in its infancy. While the

opportunities for validating causality of biomarkers (e.g. blood or image based) are immense, current findings are preliminary[24–26] and should be interpreted with caution until this field has further evolved.

Challenges and Future Perspectives

The fact that multiple genetic variants, with small effect sizes, together with environmental factors confer migraine susceptibility, has, thus far, hampered easy mapping of genetic biomarkers. While rare monogenic subtypes have been characterized in-depth[27,28], the genomic characterization of the more common migraine forms are still in its infancy. Emerging genomic information could possibly comprise better disease characterization, as is already commonly used for monogenic subtypes for which genetic biomarkers have been identified and implemented in diagnostic criteria[29]. Also, identification of genetic risk factors might potentially guide individualized management and improve clinical outcome. A recent small proof-of-concept study suggested a correlation of high migraine polygenic risk score with better treatment response to triptans , providing a first small step toward genetics-based precision medicine.[30] Further exploration of the clinical use of pharmaco-genetics requires large, prospective (epi)genetic studies focusing on treatment response with in depth treatment response data with clear definition of effectiveness, as well as large groups of (non-) responders.

Provocation Biomarkers [695 words]

The pathogenesis of migraine is multifaceted, with a complex interplay between different molecular signaling pathways.[5] A key feature of migraine is that various trigger factors are associated with an increased probability of attacks.[31] This provides a unique opportunity to identify signaling pathways underlying migraine pathogenesis through human provocation models, wherein endogenous signaling molecules or other putative 'trigger' molecules are used to induce migraine in humans.[31] An important

observation from human provocation studies is that only individuals with migraine develop provoked migraine attacks, whereas healthy volunteers only develop a mild featureless headache.[31]

In principle, human provocation models apply a double-blind, crossover design in which individuals with migraine or healthy volunteers are randomly allocated to receive a putative 'trigger' molecule or placebo.[31] A headache diary is used to record headache occurrence as well as its characteristics and accompanying symptoms.[31] Of note, provoked migraine attacks are defined as either (1) a headache that fulfils ICHD-3 criteria C and D for migraine without aura[5] or (2) a headache that mimics the respective study participants' usual migraine and is treatable with an acute medication for migraine.[5]

The first migraine provocation study demonstrated that individuals with migraine were more likely to develop headache than healthy volunteers following intravenous administration of the nitric oxide donor, glyceryl trinitrate (GTN).[32] Since then, various putative 'trigger' molecules have been tested for their ability to induce migraine, including calcitonin gene-related peptide (CGRP)[33], pituitary adenylate cyclase-activating polypeptide (PACAP)[34,35], vasoactive intestinal peptide (VIP)[36], phosphodiesterase 3 and 5 inhibitors[37,38] as well as an adenosine triphosphate-sensitive potassium channel opener (K_{ATP}).[39]

Intravenous infusion of CGRP or PACAP induces migraine attacks in approximately two-thirds of individuals with migraine.[33–35] Higher induction rates (≥80%) have been observed following administration of GTN and phosphodiesterase 3 and 5 inhibitors.[32,37,38] A common denominator for all of these '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).[40] Based on these findings, it was hypothesized that downstream effects of cAMP and cGMP signaling may involve modulation of ion channels.[41,42] Indeed, it was subsequently demonstrated that administration of a K_{ATP} channel opener yielded a migraine induction rate of 100% in individuals with migraine.[39]

Challenges and Future Perspectives

Human provocation models have provided enormous insight into signalling pathways underlying migraine pathogenesis. In addition, these studies have contributed to identification and development of drugs targeting specific 'trigger' molecules. An excellent example is the recently approved monoclonal antibodies (mAbs) targeting CGRP or its receptor that have proven effective for preventive treatment of migraine[43] and the CGRP small molecule antagonists that are effective for the acute treatment of migraine and are under investigation for migraine prevention. As a result, future drug development should in part be guided by discoveries from human provocation studies. From this perspective, one highly intriguing drug target is K_{ATP} channel blockers, as opening of these channels provoked migraine attacks in all participants.[39] However, there are also pitfalls in human provocation models.[44] For example, GTN induces migraine attacks[31] and migraine patients report headache relief after administration of non-selective nitric oxide synthase (NOS) inhibitor.[45] Based on these data NOS inhibition has been suggested as a novel target for migraine therapy. However, inducible nitric oxide (iNOS) inhibition failed to abort or prevent migraine attacks.[46,47] Nonetheless, selective inhibition of other NOS isoforms – endothelial NOS (eNOS) and neuronal NOS (nNOS) – might still prove to be useful drug targets, with promising data from preclinical pain trials using selective nNOS inhibition.[48]

Apart from discovery of drug targets for migraine, human provocation models may also be used as a biomarker to predict efficacy of mechanism-based therapies, such as the anti-CGRP mAbs.[49] To achieve this, 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 anti-CGRP mAbs. The hypothesis is that individuals with migraine who develop provoked migraine attacks following CGRP infusion would benefit more from treatment with anti-CGRP Abs than those who did not develop provoked attacks after CGRP infusion. If successful, human provocation models could provide the first predictive biomarker of treatment response in individuals with migraine.

Blood Biomarkers [622 words]

Research into blood biomarkers of migraine has garnered considerable attention over the past decades.[50] This interest has been fueled by the concept of blood biomarkers providing a window into the molecular mechanisms underlying migraine. Efforts have also been made to establish blood biomarkers that could predict and monitor treatment response in individual patients. To date, blood biomarker studies have mainly focused on various circulating signaling molecules implicated in migraine pathophysiology.

Ictal Biomarkers – during migraine attacks

In 1990, the first study investigated plasma levels of CGRP in the external jugular vein during spontaneous migraine attacks.[51,52] It was demonstrated that CGRP plasma levels were elevated in individuals with migraine, compared with historical non-headache controls. Subsequently, another study reported that ictal plasma levels of CGRP were also elevated in peripheral blood.[53] However, these findings were not reproducible in a rigorous validation study that assessed CGRP plasma concentrations in both the external jugular vein and peripheral blood, using two different assays.[54]

Ictal changes of PACAP and VIP have also been investigated. In both the external jugular vein and peripheral blood, elevated PACAP-like immunoreactivity was reported during spontaneous migraine attacks[53,55], whereas no ictal increases were found for VIP, except in a small subset of patients with prominent autonomic symptoms.[54,56]

Interictal Biomarkers – between migraine attacks

Measurements of blood biomarkers have also been performed outside of migraine attacks in both individuals with episodic and chronic migraine. The available data is highly conflicting, with strikingly different findings. Two studies have reported elevated interictal plasma levels of CGRP in individuals with both episodic and chronic migraine, compared with healthy controls.[57,58] However,

these findings were not reproduced by another study that found no differences in serum CGRP levels between individuals with chronic migraine, those with episodic migraine, and healthy controls.[59] Likewise, one study has found increased levels of VIP in both individuals with episodic and chronic migraine, whereas another study found no increase in those with episodic migraine, compared with healthy controls.[60,61] In terms of PACAP, three studies have found no increases in the interictal phase of migraine.[53,61,62]

Biomarker-Guided Therapy - prediction of treatment response

Two studies have reported higher baseline concentrations of CGRP in individuals with migraine who subsequently benefited from preventive treatment with botulinum toxin A compared to those who did not receive therapeutic benefit.[63,64] However, this finding was not reproduced in a subsequent validation study.[59]

Other blood biomarker studies

Recently, new large-scale plasma metabolome and proteome analyses are possible with high throughput screening. As an example, in a recent study, plasma samples from over 10.000 participants were profiled on a 1H-NMR-based metabolomics platform, to quantify almost 150 individual metabolites and metabolite ratios (e.g., lipids, fatty acids, and lipoproteins), and revealed alterations in HDL metabolism in migraine.[65]

Challenges and Future Perspectives

Research into blood biomarkers of migraine is still in its infancy, with much work left to be done. A lack of standardized methods for data collection and sample processing hampers comparisons between studies. In addition, suboptimal assay validation often leads to an inability in determining whether the assay only detects the blood biomarker of interest. For example, ELISA assays are used to detect CGRP and PACAP, but these assays may also detect close relatives, such as PACAP-38 versus PACAP-27 or αCGRP versus βCGRP versus amylin (~40% identical sequence to CGRP).[66]

As such, each assay must initially be validated through a rigorous process that accounts for sensitivity, specificity, inter- and intra-assay variability as well as the effect of matrix interference (serum/plasma).[67] Aside from assay validation, much emphasis should also be placed on conducting studies with large samples and appropriate control groups. Lastly, future studies should consider a shift from single-biomarker approaches to a panel of multiple biomarkers. Such an approach might show better separation between groups and yield reproducible data needed for validation of blood-based biomarkers for migraine.

Imaging Biomarkers [602 words]

In the search for migraine biomarkers, neuroimaging studies have emphasized certain functional signatures and structural alterations characterizing the different phases of the migraine cycle. Anatomical and functional studies have been conducted either interictally (between attacks), or ictally, during migraine attacks.

Structural Imaging

Numerous studies have investigated differences in brain structure between migraine vs. healthy controls and between migraine with aura vs. without aura. In addition, a few studies have compared migraine to other headache types, such as cluster headache, tension-type headache, and post-traumatic headache. These studies have demonstrated that differences in regional volumes, cortical thickness, and white matter tract integrity that might be associated with migraine and its subtypes. For example, a population-based study reported that females with migraine with aura had a thicker cortex corresponding to visual areas.[68] A diffusion tensor imaging study found interictal alterations of the trigeminal somatosensory pathway and periaqueductal gray matter in migraine.[69] Studies that have developed imaging-based diagnostic classification models using brain structural data demonstrate feasibility for differentiating migraine from healthy controls, episodic migraine from chronic migraine, and migraine from other headache types. White matter hyperintensities (WMH) are often reported in migraine studies.[70] A metanalysis study[71] showed association between WMH and MA, but not MO compared to controls. Additionally, the analysis revealed no differences in WMH between MA and MO. A population based study found no evidence of an association between WMH and migraine with aura.[72] Thus, WMH lacks both sensitivity and specificity to be used as a biomarker for migraine or its subtypes.

Functional Imaging

Interictal Phase

Functional imaging performed during the interictal phase demonstrates that migraine is associated with differences in functional connectivity and in stimulus-induced brain activation in the pain processing and visual systems[73] [74,75] In addition, recently interictal imaging of neuroinflammation with PET/MRI[76] has revealed the presence of neuroinflammation in migraine with aura in nociception processing areas, correlated with the frequency of migraine attacks.

Interictal functional connectivity data have been used to develop diagnostic biomarkers for differentiating migraine vs. healthy controls, high frequency vs. low frequency migraine attacks, and migraine vs. other pain conditions. Similar to the structural imaging classification models, these functional models require further refinement and validation.

Pre-Ictal Phase

During the preictal phase, which starts up to 48 hours before onset of migraine headache, clinical signs such as sleep disturbances or food craving have been linked to hypothalamic activation[77], including in a study of one migraine patient scanned daily over a month.[78] Imaging studies during the aura phase of a migraine attack have revealed changes consistent with cortical spreading depression,[79,80] which is believed to be the underlying biological substrate of aura.[81]

Ictal Phase

Functional imaging studies showed increased activity within the dorsal pons during the pain phase[82,83], which contains primary afferents from the trigeminal system, together with cholinergic and noradrenergic nuclei that may be involved in pain perception and modulation.[84] This has been replicated multiple times with different imaging modalities in MA and MO[77,83,85–88], leading to the conclusion that dorsal pontine activation might be a neuroimaging biomarker for the pain phase of migraine. Increased functional connectivity between dorsal pons and primary somatosensory cortex corresponding to the head and face somatotopic areas has also been reported during pain phase in

MA.[89] In MO, Amin et al. reported altering of the network connectivity between thalamus and pain encoding and modulating cortical areas[90], whereas the diameter of extracranial arteries remained unchanged.[91]

Challenges and Future Perspectives

Structural and functional imaging studies have provided important insights into migraine pathobiology and have set the stage for development of imaging-based biomarkers. Much more work is required to refine these imaging biomarkers, improve their accuracy, determine their specificity for migraine and its subtypes, and ultimately to validate them for clinical use. Standardized imaging protocols, advanced imaging techniques with ultra-high (7-T) MRI imaging, homogeneous study samples, better characterization, and data integration are needed to define a migraine-specific biomarker.[92] To provide information beyond that which can be collected by clinical history alone, the biomarkers should differentiate amongst headache types with overlapping clinical symptoms, prognosticate patient outcomes, and predict treatment responses.

Data integration: Challenges and Opportunities [655 words]

The continued search for and validation of biomarkers with diverse applications from risk prediction and screening to diagnosis and prognosis, and the creation of specific algorithms useful in preclinical and clinical settings are encouraged. To identify and integrate promising biomarkers, several steps in the investigations are required; (a) assessing associations with preclinical and clinical phases of migraine, (b) confirming their replication in numerous studies, (c) revealing the link to high biological (i.e., sex, age, and genetic background, ethnicity, epigenetics, microbiome and environmental factors) and methodological variability, and (d) testing their effective clinical utility before affirming any

definitive statement on their potential relevance in clinical migraine management. In addition, combinations of multi-omics biomarkers (epigenomics, genomics, transcriptomics, proteomics, metabolomics, radiomic, and nutrigenomics), rather than a single biomarker, are required to improve migraine prediction, diagnosis, and prognosis by creating algorithms. To this aim, further advances may be achieved by studying, through a new technological appraisal based on innovative approaches and systems, molecules associated with disease pathways that can represent valid migraine surrogates.

Data Integration of Biomarker Modalities

A combination of genetic and provocation biomarker modalities has been used to investigate the effects of CGRP in individuals with FHM. Interestingly, CGRP did not induce migraine attacks in both FHM patients who had known ion channel mutations[95] as well as those who did not.[96] These findings indicate that individuals with FHM do not display hypersensitivity to CGRP which differs from data in individuals with common types of migraine.[33,97] In addition, another provocation study found no association between high family load (≥ 2 first-degree relatives with migraine) and migraine induction following PACAP infusion in individuals with migraine without aura.[98]

Another combination of modalities includes neuroimaging and human provocation models. Three studies have used 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 MCA and MMA.[99] Interestingly, MCA and MMA dilation were only present on the pain side in those who developed unilateral migraine attacks.[99] In another MRA study, MCA and MMA changes were recorded after migraine induction using a phosphodiesterase 3 inhibitor.[100] The authors reported that the provoked attacks were associated with an MMA dilation on the pain side, but no dilation of the MCA. Lastly, another MRA study found that PACAP-induced migraine attacks were associated with MMA dilation, but not MCA dilation.[101] In addition, the authors found no association between provoked attacks and pain location. Another

option is to combine neuroimaging and provocation model to examine changes in functional connectivity before and at onset of provoked migraine attacks. In one randomized, double-blind rs-fMRI study, abnormal functional connectivity was found in all investigated cerebral networks (sensorimotor, salience, and default mode) following intravenous infusion of PACAP.[102] No changes in functional connectivity were found after intravenous infusion of VIP (active placebo). Interestingly, all of the investigated cerebral networks had previously been implicated in processing of nociception and emotions .[103,104]

Machine Learning and Big Data Solutions

The integration of biomarkers from multiple pathophysiological modalities into an understandable format to ensure that it is clinically useful is a major challenge not only in migraine. Despite that migraine biomarkers are in their infancy, identifying research and clinically important parameters using computational and informatics techniques, is unavoidable and potentially rewarding, but challenging. To process high-dimensional data, the field of machine learning has established a statistical and computational technique so-called big data solution. Improved characterization and classification of migraine will, ultimately, require integration of information not only from clinical methods, but also from a range of sources including genetic, blood, human models and neuroimaging biomarkers. Such integration of information will be a considerable endeavor but has the potential to enable classification of migraine patients into subgroups with more homogeneous pathophysiological mechanisms for targeted trials of novel specific interventions. This approach depends on highly accurate clinical phenotyping coupled with access to large data sources, which require inter-disciplinary and intercentre collaboration

References

1. Zunt, J. R. *et al.* Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **17,** 1061–1082 (2019).
2. Gormley, P. *et al.* Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. *Nat. Genet.* **48,** 856–866 (2016).
3. Goadsby, P. J. *et al.* Pathophysiology of migraine: A disorder of sensory processing. *Physiol. Rev.* **97,** 553–622 (2017).
4. Ashina, M. *et al.* Migraine and the trigeminovascular system—40 years and counting. *Lancet Neurol.* **4422,** 1–10 (2019).
5. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. *Cephalalgia* **38,** 1–211 (2018).
6. Silberstein, S. D. Migraine Symptoms: Results of a Survey of Self-Reported Migraineurs. *Headache J. Head Face Pain* **35,** 387–396 (1995).
7. Russell, M. B., Rasmussen, B. K., Fenger, K. & Olesen, J. Migraine without aura and migraine with aura are distinct clinical entities: A study of four hundred and eighty-four male and female migraineurs from the general population. *Cephalalgia* **16,** 239–245 (1996).
8. Russell, M. B. & Olesen, J. A nosographic analysis of the migraine aura in a general population. *Brain* **119,** 355–361 (1996).
9. Hansen, J. M. *et al.* Migraine headache is present in the aura phase : A prospective study. *Neurology* **79,** 2044–2049 (2012).
10. Chalmer, M. A. *et al.* Proposed new diagnostic criteria for chronic migraine. *Cephalalgia* **0,** 1–8 (2019).
11. Polderman, T. J. C. *et al.* Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nat. Genet.* **47,** 702–9 (2015).
12. Russell, M. B. & Olesen, J. Increased Familial Risk And Evidence Of Genetic Factor In Migraine. *BMJ* **311,** 541–544 (1995).
13. Russell, M. B., Ulrich, V., Gervil, M. & Olesen, J. Migraine without aura and migraine with aura are distinct disorders. A population-based twin survey. *Headache* **42,** 332–336 (2002).
14. Winsvold, B. S. *et al.* Genetic analysis for a shared biological basis between migraine and coronary artery disease. *Neurol. Genet.* **1,** e10 (2015).
15. Malik, R. *et al.* Shared genetic basis for migraine and ischemic stroke: A genome-wide analysis of common variants. *Neurology* **84,** 2132–45 (2015).
16. Gormley, P. *et al.* Common Variant Burden Contributes to the Familial Aggregation of Migraine in 1,589 Families. *Neuron* **98,** 743–753.e4 (2018).
17. Pelzer, N. *et al.* Linking migraine frequency with family history of migraine. *Cephalalgia* **39,** 229–236 (2019).
18. Gerring, Z. F., McRae, A. F., Montgomery, G. W. & Nyholt, D. R. Genome-wide DNA methylation profiling in whole blood reveals epigenetic signatures associated with migraine. *BMC Genomics* **19,** 1–10 (2018).

19. De Boer, I., Van Den Maagdenberg, A. M. J. M. & Terwindt, G. M. Advance in genetics of migraine. *Curr. Opin. Neurol.* **32,** 413–421 (2019).

20. Ferrari, M. D., Klever, R. R., Terwindt, G. M., Ayata, C. & van den Maagdenberg, A. M. J. M. Migraine pathophysiology: Lessons from mouse models and human genetics. *Lancet Neurol.* **14,** 65–80 (2015).

21. Pelzer, N. *et al.* Clinical spectrum of hemiplegic migraine and chances of finding a pathogenic mutation. *Neurology* **90,** e575–e582 (2018).

22. Emdin, C. A., Khera, A. V. & Kathiresan, S. Mendelian randomization. *JAMA - J. Am. Med. Assoc.* **318,** 1925–1926 (2017).

23. Kamstrup, P. R., Tybjærg-hansen, A., Steffensen, R. & Nordestgaard, B. G. Genetically Elevated Lipoprotein (a). *J. Am. Med. Assoc.* **301,** 2331–2339 (2009).

24. Yin, P. *et al.* Serum calcium and risk of migraine: A Mendelian randomization study. *Hum. Mol. Genet.* **26,** 820–828 (2017).

25. Daghlas, I., Guo, Y. & Chasman, D. I. Effect of genetic liability to migraine on coronary artery disease and atrial fibrillation: a Mendelian randomization study. *Eur. J. Neurol.* **27,** 550–556 (2020).

26. Johnsen, M. B. *et al.* The causal role of smoking on the risk of headache. A Mendelian randomization analysis in the HUNT study. *Eur. J. Neurol.* **25,** 1148-e102 (2018).

27. Ophoff, R. A. *et al.* Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell* **87,** 543–552 (1996).

28. De Fusco, M. *et al.* Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump α2 subunit associated with familial hemiplegic migraine type 2. *Nat. Genet.* **33,** 192–196 (2003).

29. Vincent, M. & Wang, S. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. *Cephalalgia* **38,** 1–211 (2018).

30. Kogelman, L. J. A. *et al.* Migraine polygenic risk score associates with efficacy of migraine-specific drugs. *Neurol. Genet.* **5,** e364 (2019).

31. Ashina, M., Hansen, J. M., á Dunga, B. O. & Olesen, J. Human models of migraine — short-term pain for long-term gain. *Nat. Rev. Neurol.* **13,** 713–724 (2017).

32. Olesen, J., Iversen, H. K. & Thomsen, L. L. Nitic oxide supersensitivity: a possible molecular mechanism of migraine pain. *NeuroRepost* **4,** 1027–1030 (1993).

33. Lassen, L. H. *et al.* CGRP may play a causative role in migraine. *Cephalalgia* **22,** 54–61 (2002).

34. Schytz, H. W. *et al.* PACAP38 induces migraine-like attacks in patients with migraine without aura. *Brain* **132,** 16–25 (2009).

35. Ghanizada, H., Al-Karagholi, M. A.-M., Arngrim, N., Olesen, J. & Ashina, M. PACAP27 induces migraine-like attacks in migraine patients. *Cephalalgia* **0,** 33310241986450 (2019).

36. Rahmann, A. *et al.* Vasoactive intestinal peptide causes marked cephalic vasodilation, but does not induce migraine. *Cephalalgia* **28,** 226–236 (2008).

37. Guo, S., Olesen, J. & Ashina, M. Phosphodiesterase 3 inhibitor cilostazol induces migraine-like attacks via cyclic AMP increase. *Brain* **137,** 2951–2959 (2014).

38. Kruuse, C., Thomsen, L. L., Birk, S. & Olesen, J. Migraine can be induced by sildenafil without changes in middle cerebral artery diameter. *Brain* **126,** 241–247 (2003).

39. Al-Karagholi, M. A.-M., Hansen, J. M., Guo, S., Olesen, J. & Ashina, M. Opening of ATP-sensitive potassium channels causes migraine attacks: a new target for the treatment of migraine. *Brain* **142,** 2644–2654 (2019).

40. Younis, S. *et al.* Investigation of distinct molecular pathways in migraine induction using calcitonin gene-related peptide and sildenafil. *Cephalalgia* **0,** 1–13 (2019).

41. Al-Karagholi, M. A., Christian, G., Nielsen, C. A. aldorff & Ashina, M. Targeting - BK Ca Channels in Migraine : Rationale and Perspectives. *CNS Drugs* (2020). doi:10.1007/s40263-020-00706-8

42. Al-Karagholi, M. A.-M., Hansen, J. M., Severinsen, J., Jansen-Olesen, I. & Ashina, M. The KATP channel in migraine pathophysiology: a novel therapeutic target for migraine. *J. Headache Pain* **18,** 90 (2017).

43. Do, T. P., Guo, S. & Ashina, M. Therapeutic novelties in migraine: New drugs, new hope? *J. Headache Pain* **20,** 1–13 (2019).

44. Ashina, M., Hansen, J. M. & Olesen, J. Pearls and pitfalls in human pharmacological models of migraine: 30 years' experience. *Cephalalgia* **33,** 540–53 (2013).

45. Lassen, L. H., Ashina, M., Christiansen, I., Ulrich, V. & Olesen, J. Nitric oxide synthase inhibition in migraine. *Lancet* **349,** 401–402 (1997).

46. Høivik, H. O. *et al.* Lack of efficacy of the selective iNOS inhibitor GW274150 in prophylaxis of migraine headache. *Cephalalgia* **30,** 1458–1467 (2010).

47. Van Der Schueren, B. J. *et al.* Does the unfavorable pharmacokinetic and pharmacodynamic profile of the iNOS inhibitor GW273629 lead to inefficacy in acute migraine? *J. Clin. Pharmacol.* **49,** 281–290 (2009).

48. Pradhan, A. A., Bertels, Z. & Akerman, S. Targeted Nitric Oxide Synthase Inhibitors for Migraine. *Neurotherapeutics* **15,** 391–401 (2018).

49. Christensen, C. E. *et al.* Migraine induction with calcitonin gene-related peptide in patients from erenumab trials. *J. Headache Pain* **19,** (2018).

50. Ferroni, P. *et al.* Circulating Biomarkers in Migraine: New Opportunities for Precision Medicine. *Curr. Med. Chem.* **26,** 6191–6206 (2019).

51. Goadsby, P. J., Edvinsson, L. & Ekman, R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann. Neurol.* **28,** 183–187 (1990).

52. Goadsby, P. J. & Edvinsson, L. The Trigermnovascular System and Migraine : Studies Characteruing Cerebrovascular and Neuropeptide Changes Seen in Humans and Cats. *Ann. Neurol.* **33,** 48–56 (1993).

53. Tuka, B. *et al.* Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients. *Cephalalgia* **33,** 1085–1095 (2013).

54. Tvedskov, J. F. *et al.* No increase of calcitonin gene-related peptide in jugular blood during migraine. *Ann. Neurol.* **58,** 561–568 (2005).

55. Zagami, A. S., Edvinsson, L. & Goadsby, P. J. Pituitary adenylate cyclase activating polypeptide and migraine. *Ann. Clin. Transl. Neurol.* **1,** 1036–40 (2014).

56. Sarchielli, P. *et al.* Clinical-biochemical correlates of migraine attacks in rizatriptan responders and non-responders. *Cephalalgia* **26,** 257–265 (2006).

57. Ashina, M., Bendtsen, L., Jensen, R., Schifter, S. & Olesen, J. Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. *Pain* **86,** 133–138 (2000).

58. Cernuda-Morollón, E. *et al.* Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. *Neurology* **81,** 1191–1196 (2013).

59. Lee, M. J., Lee, S. Y., Cho, S., Kang, E. S. & Chung, C. S. Feasibility of serum CGRP measurement as a biomarker of chronic migraine: a critical reappraisal. *J. Headache Pain* **19,** (2018).

60. Cernuda-Morollón, E. *et al.* Increased VIP levels in peripheral blood outside migraine attacks as a potential biomarker of cranial parasympathetic activation in chronic migraine. *Cephalalgia* **35,** 310–316 (2015).

61. Guo, S. *et al.* Part II: Biochemical changes after pituitary adenylate cyclase-activating polypeptide-38 infusion in migraine patients. *Cephalalgia* **37,** 136–147 (2017).

62. Cernuda-Morollón, E. *et al.* No Change in Interictal PACAP Levels in Peripheral Blood in Women With Chronic Migraine. *Headache* **56,** 1448–1454 (2016).

63. Cernuda-Morollõn, E. *et al.* CGRP and VIP levels as predictors of efficacy of onabotulinumtoxin type A in chronic migraine. *Headache* **54,** 987–995 (2014).

64. Domínguez, C. *et al.* CGRP and PTX3 as Predictors of Efficacy of Onabotulinumtoxin Type A in Chronic Migraine: An Observational Study. *Headache* **58,** 78–87 (2018).

65. Onderwater, G. L. J. *et al.* Large-scale plasma metabolome analysis reveals alterations in HDL metabolism in migraine. *Neurology* **92,** E1899–E1911 (2019).

66. Hay, D. L., Garelja, M. L., Poyner, D. R. & Walker, C. S. Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR Review 25. *Br. J. Pharmacol.* **175,** 3–17 (2018).

67. U.S. Department of Health and Human Services. Food and Drug Administration. Guidance for Industry: Bioanalytical Method Validation. 4–10 (2018). doi:http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-4252fnl.pdf

68. Gaist, D. *et al.* Migraine with visual aura associated with thicker visual cortex. *Brain* **141,** 776–785 (2018).

69. DaSilva, A. F. M. *et al.* Interictal alterations of the trigeminal somatosensory pathway and periaqueductal gray matter in migraine. *Neuroreport* **18,** 301–305 (2007).

70. Palm-Meinders, I. H. *et al.* Structural brain changes in Migraine. *JAMA* **308,** 1889–1897 (2013).

71. Bashir, A., Lipton, R., Ashina, S. & Ashina, M. Migraine and structural changes in the brain. *Neurology* **81,** 1260–1268 (2013).

72. Uggetti, C. *et al.* Migraine with aura and white matter lesions: an MRI study. *Neurol. Sci.* **38,** 11–13 (2017).

73. Mainero, C., Boshyan, J. & Hadjikhani, N. Altered functional magnetic resonance imaging resting-state connectivity in periaqueductal gray networks in migraine. *Ann. Neurol.* **70,** 838–

845 (2011).

74. Hougaard, A. *et al.* No abnormalities of intrinsic brain connectivity in the interictal phase of migraine with aura. *Eur. J. Neurol.* **22,** 702-e46 (2015).

75. Skorobogatykh, K. *et al.* Functional connectivity studies in migraine: what have we learned? *J. Headache Pain* **20,** (2019).

76. Albrecht, D. S. *et al.* Imaging of neuroinflammation in migraine with aura: A [11C]PBR28 PET/MRI study. *Neurology* **92,** E2038–E2050 (2019).

77. Maniyar, F. H., Sprenger, T., Monteith, T., Schankin, C. & Goadsby, P. J. Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks. *Brain* **137,** 232–241 (2014).

78. Schulte, L. H. & May, A. The migraine generator revisited: continuous scanning of the migraine cycle over 30 days and three spontaneous attacks. *Brain A J. Neurol.* **139,** 1987–1993 (2016).

79. Hadjikhani, N. *et al.* Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 4687–4692 (2001).

80. Olesen, J. *et al.* Timing and topography of cerebral blood flow, aura, and headache during migraine attacks. *Ann. Neurol.* **28,** 791–798 (1990).

81. Ayata, C. & Lauritzen, M. Spreading depression, spreading depolarizations, and the cerebral vasculature. *Physiol. Rev.* **95,** 953–993 (2015).

82. Weiller, C. *et al.* Brain stem activation in spontaneous human migraine attacks. *Nat. Med.* **1,** 25–27 (1995).

83. Afridi, S. K. *et al.* A positron emission tomographic study in spontaneous migraine. *Arch. Neurol.* **62,** 1270–1275 (2005).

84. Borsook, D. & Burstein, R. The enigma of the dorsolateral pons as a migraine generator. *Cephalalgia* **32,** 803–812 (2012).

85. Stankewitz, A., Aderjan, D., Eippert, F. & May, A. Trigeminal nociceptive transmission in migraineurs predicts migraine attacks. *J. Neurosci. Off. J. Soc. Neurosci.* **31,** 1937–1943 (2011).

86. Hougaard, A. *et al.* Increased brainstem perfusion, but no blood-brain barrier disruption, during attacks of migraine with aura. *Brain A J. Neurol.* **140,** 1633–1642 (2017).

87. Denuelle, M., Fabre, N., Payoux, P., Chollet, F. & Geraud, G. Hypothalamic activation in spontaneous migraine attacks. *Headache* **47,** 1418–1426 (2007).

88. Younis, S. *et al.* Glutamate levels and perfusion in pons during migraine attacks: a 3 tesla MRI study using proton spectroscopy and arterial spin labeling. *J. Cereb. Blood Flow Metab.* In press (2020).

89. Hougaard, A., Amin, F. M., Larsson, H. B. W., Rostrup, E. & Ashina, M. Increased intrinsic brain connectivity between pons and somatosensory cortex during attacks of migraine with aura. *Hum. Brain Mapp.* **38,** 2635–2642 (2017).

90. Amin, F. M. *et al.* Altered thalamic connectivity during spontaneous attacks of migraine without aura: A resting-state fMRI study. *Cephalalgia* **38,** 1237–1244 (2018).

91. Amin, F. M. *et al.* Magnetic resonance angiography of intracranial and extracranial arteries

in patients with spontaneous migraine without aura: A cross-sectional study. *Lancet Neurol.* **12,** 454–461 (2013).

92. Hougaard, A., Amin, F. M. & Ashina, M. Migraine and structural abnormalities in the brain. *Curr. Opin. Neurol.* **27,** 309–314 (2014).

93. Schwedt, T. J. *et al.* Accurate classification of chronic migraine via brain magnetic resonance imaging. *Headache* **55,** 762–777 (2015).

94. Chong, C. D. *et al.* Migraine classification using magnetic resonance imaging resting-state functional connectivity data. *Cephalalgia* **37,** 828–844 (2017).

95. Hansen, J. M., Thomsen, L. L., Olesen, J. & Ashina, M. Calcitonin geneg related peptide does not cause the familial hemiplegic migraine phenotype. *Neurology* **71,** 841–847 (2008).

96. Hansen, J. M., Thomsen, L. L., Olesen, J. & Ashina, M. Calcitonin gene-related peptide does not cause migraine attacks in patients with familial hemiplegic migraine. *Headache* **51,** 544–553 (2011).

97. Hansen, J. M., Hauge, A. W., Olesen, J. & Ashina, M. Calcitonin gene-related peptide triggers migraine-like attacks in patients with migraine with aura. *Cephalalgia* **30,** 1179–1186 (2010).

98. Guo, S. *et al.* Part I: Pituitary adenylate cyclase-activating polypeptide-38 induced migraine-like attacks in patients with and without familial aggregation of migraine. *Cephalalgia* **37,** 125–135 (2016).

99. Asghar, M. S. *et al.* Evidence for a vascular factor in migraine. *Ann. Neurol.* **69,** 635–645 (2011).

100. Khan, S. *et al.* Meningeal contribution to migraine pain: a magnetic resonance angiography study. *Brain* **142,** 93–102 (2019).

101. Amin, F. M. *et al.* Investigation of the pathophysiological mechanisms of migraine attacks induced by pituitary adenylate cyclase-activating polypeptide-38. *Brain* **137,** 779–94 (2014).

102. Amin, F. M. *et al.* Change in brain network connectivity during PACAP38-induced migraine attacks. *Neurology* **86,** 180–187 (2016).

103. Borsook, D., Edwards, R., Elman, I., Becerra, L. & Levine, J. Pain and analgesia: The value of salience circuits. *Prog. Neurobiol.* **104,** 93–105 (2013).

104. Seeley, W. W. *et al.* Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* **27,** 2349–2356 (2007).

Figure 1. Migraine biomarkers. Advances in migraine-specific biomarkers hail from genetics, provocation models, biochemistry, and neuroimaging.

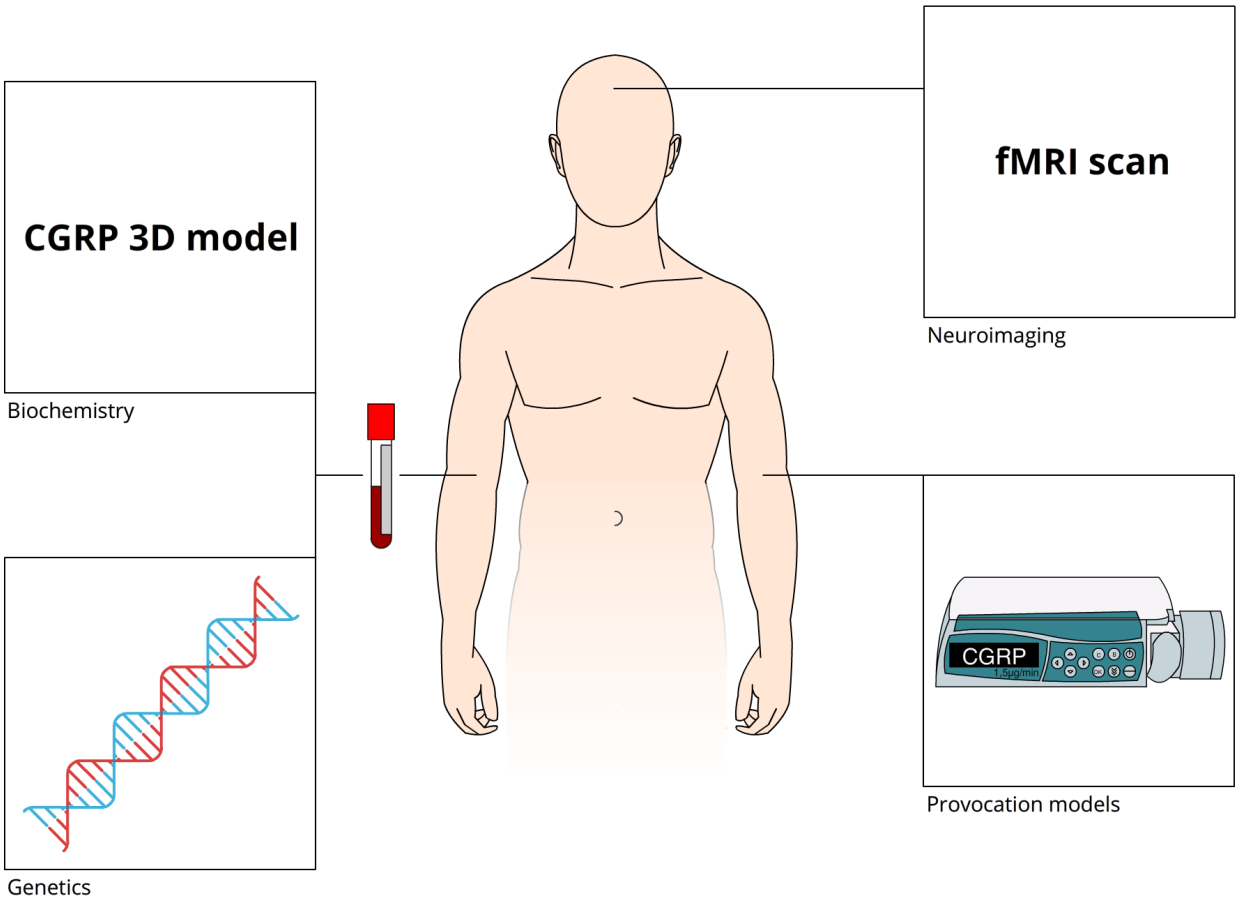

Figure 2. Genetic biomarkers. Emerging genomic information could possibly comprise better disease characterization, risk identification, guide individualized management and improve clinical outcome.

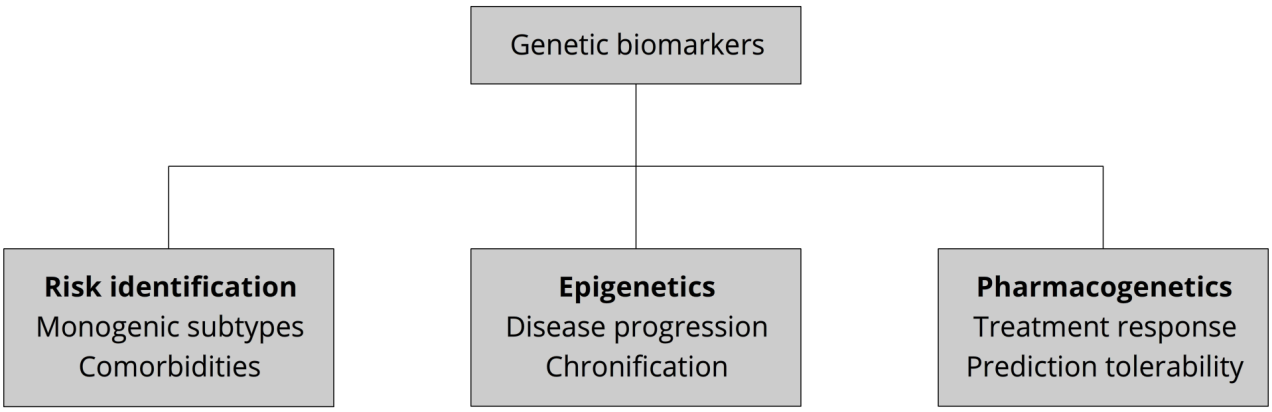

Figure 3. Molecular signaling pathways in migraine. The migraine induction pathways of migraine have been outlined by the use of various putative 'trigger' molecules, including calcitonin gene-related peptide (CGRP; ~60% induction rate), pituitary adenylate cyclase-activating polypeptide (PACAP); ~60% induction rate, phosphodiesterase 3 and 5 inhibitors (cilostazol; ~90% and sildenafil; ~80% induction rate) as well as an adenosine triphosphate-sensitive potassium channel opener (K_{ATP}; ~100% induction rate).

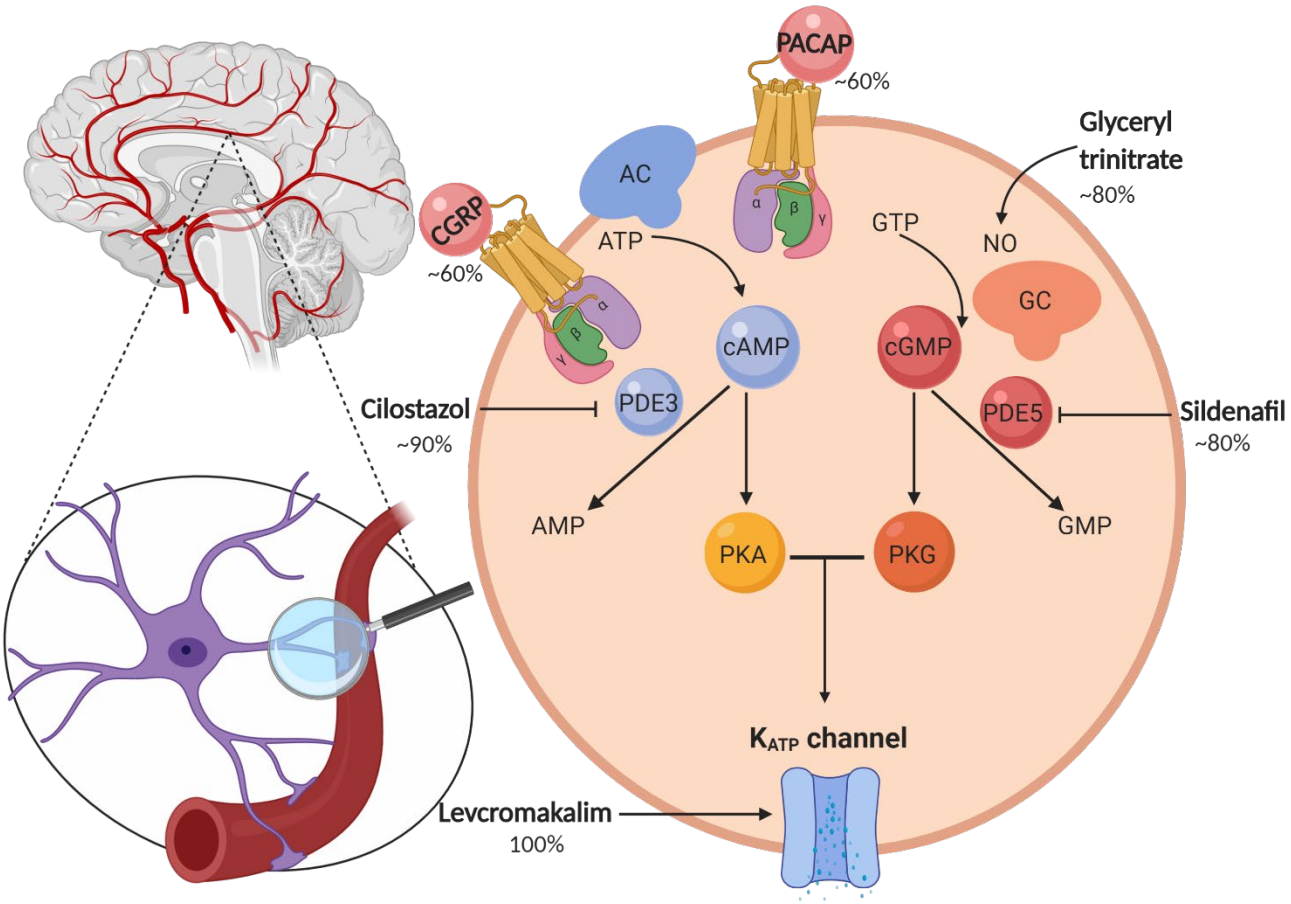

Figure 4. Functional activity the migraine brain. The preictal phase before a headache attack have been linked to hypothalamic activation while studies showed increased activity within the dorsal pons during the pain phase. During the aura phase of a migraine attack, cerebral blood flow imaging studies have revealed changes consistent with cortical spreading depression.