Unravelling the mystery of migraine and cluster headache: insights into the genetics and biochemistry of these neurological disorders

Harder, A.V.E.

General introduction

Adapted from:
Migraine genetics: Status and road forward
Aster V.E. Harder, Gisela M. Terwindt, Dale R. Nyholt and Arn M.J.M. van den Maagdenberg

Cephalalgia. 2023;43:3331024221145962

1

Introduction

This thesis aims to further unravel the pathophysiology of migraine and cluster headache. Both migraine and cluster headache are disabling primary headache disorders characterized by attacks of severe headache and associated symptoms.[1] Cluster headache is one of the trigeminal autonomic cephalalgias.[1] By definition, primary headache disorders are not the result of any other underlying disease or process, contrary to secondary headache disorders. Although much progress has been made with unravelling the disease mechanisms of migraine and cluster headache, their pathophysiology remains poorly understood.[2, 3] A major hurdle is that there are no diagnostic biomarkers and the diagnosis, therefore, is still made using direct interviews and/or questionnaires based on clinical consensus criteria of the International Classification of Headache disorders (ICHD-3 criteria).[1] A shortcoming of the current classification criteria is that it does not take the complexity of disease mechanisms into account. In other words, the ICHD-3 criteria do not fully capture the heterogeneity of the disease, including the underlying neurobiological and genetic factors.[4] Understanding the pathophysiology better will improve diagnosis, prognosis, and generate new treatment options.

Clinical characteristics

Migraine

Migraine is characterized by recurrent episodes of severe often unilateral pulsating headache accompanied by nausea, vomiting and/or photo- and phonophobia lasting for 4-72 hours.[1] Migraine can be subdivided in two main subtypes: migraine without aura and migraine with aura. For the latter, headaches are preceded by transient neurological symptoms, known as the aura phase, which typically lasts from 5 until 60 minutes.[1] Cortical spreading depolarization (CSD) is the presumed underlying mechanism of the aura in migraine.[5-8] A typical migraine attack consist of a preictal, ictal (aura and/or headache), and postictal (postdromal) phase.[9, 10] Clinically, a patient can be described as interictal, when there is no attack or ictal when an aura and/or migrainous headache is occurring. Migraine is three times more prevalent in women than in men with a peak prevalence of 25%.[11, 12] Migraine is associated with several neuropsychiatric disorders, among which depression.[13] Migraine is considered a multifactorial (complex) genetic disorder, with a strong familial aggregation.[14-16] Complex traits are typically brought about by a combination of multiple genetic variants, each with a small effect size, and behavioural and environmental factors. Hemiplegic migraine (HM) is a rare subtype of migraine with aura, HM is characterized by attacks that are associated with motor weakness that can lead to hemiplegia during the aura phase.[1]

Cluster headache

Cluster headache is a primary headache disorder characterized by excruciating unilateral headache or facial pain accompanied by ipsilateral facial autonomic symptoms and/or restlessness.[1,17] Attacks

may last for 15-180 minutes and can occur from once a day up to 8 times or more a day.[1] Cluster headache attacks commonly follow a circadian rhythm with attacks frequently occurring at night and according to a seasonal pattern. The majority of patients have episodic cluster headache, characterized by periods of cluster headache of weeks to months, alternating with attack-free periods of at least 3 months. A small proportion (10-15%) of patients have chronic cluster headache where the cluster periods do not remit for more than three months for at least one year. Cluster headache has a prevalence of around 0.12% and occurs more often in men than women, with a male-to-female ratio of 2:1.[18, 19] Of note, smoking and psychiatric co-morbidities are prevalent among cluster headache patients.[20] The pathophysiology of cluster headache is poorly understood with current evidence pointing at hypothalamic involvement.[3] Genetic predisposition seems to play an important role as illustrated by twin and family studies but no genetic factors have been identfied.[21]

Pathophysiology

Migraine

Different disease mechanisms are considered to be involved in migraine pathophysiology, such as neurological, cerebrovascular, and neuroinflammatory mechanisms. The aura phase is most likely caused by CSD, a wave of neuronal and glial depolarization, that is an initial hyperactivity is followed by a prolonged inactivity, resulting in a wave that propagates slowly across the cerebral cortex.[22, 23] The depolarization wave classically begins in the occipital (visual) cortex and correlates with a variety of positive aura patterns, as reported by patients.[24, 25] Mechanisms of CSD are heavily investigated in animals using various stimuli, such as topical application of KCl, injection of current, or an optogenetic stimulus, and it was shown that CSD can activate headache mechanisms.[26] However, there is only limited (neuroimaging) data that can be taken as proof of a spreading depolarization event that qualifies as an aura in humans.[27] Also whether the CSD is causally associated with the initiation of the headache phase in patients remains an enigma.[28]

It is generally accepted that the headache phase involves the activation and sensitization of the trigeminovascular system.[29] The trigeminovascular system consists of nociceptive trigeminal afferents from the trigeminal ganglion that surround cranial blood vessels and dura mater projected from the trigeminal cervical complex in the brainstem, which includes the trigeminal nucleus caudalis and the dorsal horns of cervical spinal nerves C1 and C2.[30] Following stimulation, the trigeminal afferents transfer nociceptive signals through the trigeminal ganglion to the trigeminal cervical complex. In the brainstem, the signal is modulated and further conducted to the thalamus via ascending pain pathways and reaches the cortex.[25] Upon stimulation, the trigeminal fibres release proinflammatory neuropeptides (e.g. calcitonin-gene related peptide (CGRP), pituitary adenylate cyclase-activating peptide (PACAP) substance P and neurokinin A) and other mediators that cause vasodilation of the dural and pial vessels.[25] There is ample evidence that vasodilators such as

prostaglandins may be pivotal in the development of migraine attacks.[7,31,32] Increased sensitivity of the trigeminal system is believed to be an important underlying mechanism in migraine pathology. The mechanisms underlying this hypersensitivity during a migraine attack remain unclear.

Additionally, the hypothalamus is believed to be involved in the prodromes (symptoms that precede the migraine headache).[33] Clinically this is evident by increased fatigue, food cravings, yawning and irritability in the patients. Therefore the phase before the migraine attack can also give insights into the pathophysiology of migraine.

Cluster headache

Various mechanisms/structures, e.g. the trigeminovascular system and the hypothalamus, are believed to be involved in cluster headache pathophysiology and it is thought that the interplay of these systems is responsible for the clinical presentation.[34,35] However, how these structures interact with each other and the mechanisms on the initiation of an attack remain unclear. Similar to migraine, also in cluster headache the trigeminovascular system is believed to be involved in pain processing.[34,35] Different divisions of the trigeminal nerve are primarily responsible for the innervation of cranial structures. Stimulations of the different divisions produce pain in different locations, activation of the second-order trigeminocervical neurons at the ophthalmic division is in line with the clinical presentation of pain in the peri-orbital region.[35]

The trigeminal-autonomic reflex is also associated with the physiological and anatomical landmarks of a cluster headache attack. This reflex is activated upon irritation and produces parasympathetic symptoms, such as nasal congestion and lacrimation.[36] The reflex travels from trigeminal nerve endings to second-order trigeminocervical complex, that projects to the superior salivatory nucleus located in the pons.[34,35] These projections in turn synapse in the peripheral sphenopalatine ganglion and postganglionic parasympathetic nerves and then innervate nasal, pharyngeal and lacrimal glands, inducing autonomic symptoms.[35] Activation of the trigeminovascular system and the trigeminal-autonomic reflex leads to release of neuropeptides (e.g. CGRP and PACAP).

In addition, the hypothalamus is believed to be a key player in cluster headache pathophysiology. The hypothalamus is involved in the regulation of sleep and circadian rhythms.[37] The hypothesis that the hypothalamus is involved in cluster headache is supported by the clinical feature of a circadian rhythm in cluster headache and the finding that the hypothalamus shows increased activation during glyceryl trinitrate (GTN) induced attacks of cluster headache.[38]

Rationale for biochemical studies

Identifying biochemical markers, biomarkers, can help uncover the metabolic underpinnings of human disease. Validated biomarkers can improve diagnosis, prognosis and assess the effectivity of

treatment in patients and lead to novel drug targets, and ultimately novel drugs. This has already been shown for several diseases other than migraine or cluster headache, for instance cardiac troponin helps diagnose myocardial infarction and different biomarkers have been developed for the diagnosis of ovarian cancer.[39-41] A way to investigate whether endogenous signalling molecules are involved in migraine pathology is by trying to provoke an attack in "a human model". When an attack can be provoked with a certain trigger, this suggests the involvement of a related mechanism underlying the disease. Several chemical molecules have been implicated in migraine, identified as they can trigger attacks. The triggers are mostly vasoactive substances that are present at or near the nerve fibres. It has been shown that glyceryl trinitrate (GTN), an nitric oxide (NO) donor, is able to induce an immediate headache in almost all subjects and a delayed migraine-like attack in close to 70% of migraineurs but not in controls.[42, 43] Other substances, such as calcitonin gene-related peptide (CGRP), PACAP and prostaglandin E_2 (PGE_2) and I_2 (PGI_2) are also able to trigger migraine-like attacks.[44-47] Although attacks of cluster headache have been successfully triggered with GTN and histamine, it is not common practice to investigate cluster headache using provocation studies.[48] In addition to investigating trigger mechanisms *per se*, provocation studies can also be used to study other aspects of migraine, such as consequences of attacks, as investigating spontaneous attacks is notoriously difficult as they occur unexpectedly. In contrast, in provocation studies, the set-up can be meticulously controlled.

Another way of investigating relevant substances in disease is by measuring compounds in body fluids, such as cerebrospinal fluid (CSF), blood and urine, and compare profiles in disease vs. control samples. The compounds, being proteins (proteomics) or metabolites (metabolomics), are representative of aspects of the phenotype at the molecular level. For instance, altered blood plasma levels of serotonin (5-HT) in migraine patients were found in the late eighties.[49] This finding contributed to the development of triptans, i.e. 5-HT-1D/1F receptor agonists, which are used for aborting migraine attacks. Serotonin is an amine, just like other neurotransmitters implicated in migraine pathophysiology, such as glutamate and gamma-aminobutyric acid (GABA). This led to amines to be further investigated in the pathogenesis of migraine.[50] Recently, a lot of biochemical research was done on CGRP, which is believed to play an important role in migraine and cluster headache. As mentioned earlier, infusion of CGRP is able to induce migraine-like attacks in migraine patients.[47] In addition, studies show an increase in CGRP levels in blood between cases and controls outside[51-57] or during a migraine attack,[53, 57-61] although almost as many studies have not found a difference in CGRP levels in blood outside[58, 60, 62-65] or during[59, 65] migraine attacks. Regardless, newly approved monoclonal antibodies (mAbs) that target CGRP or its receptor have a beneficial effect on the headache frequency in patients with migraine.[66, 67] In cluster headache, CGRP also seems to be involved[68, 69] with CGRP plasma levels being higher for cluster headache patients during an active period compared to those outside, after provocation with sublingual GTN.[70] In cluster headache, randomized controlled trials on CGRP antibodies in patients have been initiated but with unconvincing results sofar.[71] However, the reliability of measuring CGRP is not without controversy.[65, 72]

When identifying biochemical compounds in body fluids either using a targeted approach, which typically focuses on one or more related selected pathways of interest, or an untargeted approach, which aims to simultaneously measure a large number of metabolites, can be employed. The latter method is most commonly used in the field of metabolomics. Metabolomics is defined as the study of all low molecular weight compounds (<1500 Da) in a sample. Metabolites are the molecular endpoints of gene expression and cell activity and thereby represent, in a way, "the molecular phenotype of an organism". The various types of -omics, i.e. genomics, epigenomics, transcriptomics, proteomic and metabolomic, relate to each other (**Figure 1**). Genomics is the study of the genome at the DNA level, as does epigenomics which investigates modifications of the genome expression. Transcriptomics investigates genomic expression at the RNA level, whereas proteomics interrogates proteins. Finally, metabolomics deals with the metabolome, so the complete set of small-molecule metabolites. Logically, changes in gene expression, enzymes and environmental factors can all have an effect on the "systems biology" and metabolite concentrations.[73] The advantage of the untargeted/omics approach is that it allows for a rapid, concurrent identification and quantification of a multitude of metabolites in many samples at once. By measuring multiple metabolites, one gets a better understanding of the overall metabolomic networks involved. Important aspects and considerations of this method are the validation of the metabolites measured, validation of the used platform, as well as standardization of collection and storage methods.[74,75]

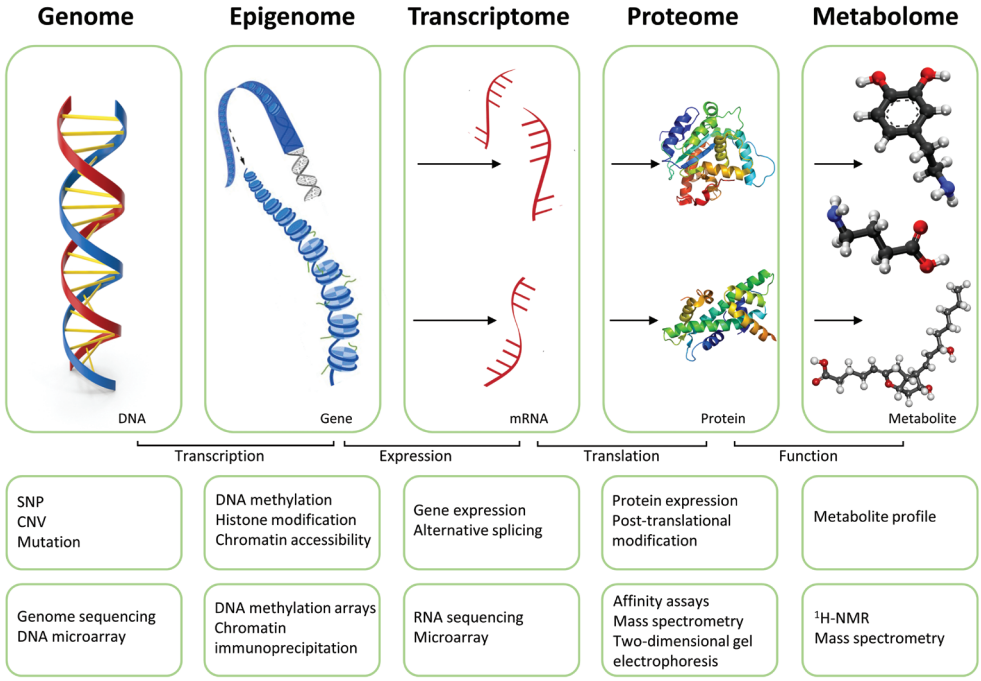
Sample collection

Although metabolomics is a proven, worthwhile approach for biomarker identification, sample collection needs to be done very meticulously as metabolically active cells in body fluids may alter the metabolomic profile *ex vivo*. It has been shown that inaccuracies in the pre-analytical steps cause low quality samples and even up to 80% of the laboratory measurement inaccuracies in daily clinical routine diagnostics.[76-78] In the field of metabolomics, the stability of many metabolites and lipids is extremely variable, therefore, systematic or pre-analytical accidents and inconsistencies can have a great effect on compounds with a low stability and lead to high variability in the analytical data. Therefore, the most critical steps regarding the quality of one's metabolomics data are related to the pre-analytical phase. Of note, each step should be well-considered, standardized and controlled to prevent degradation of sample quality and misinterpretation of findings during the analysis of data.

Important issues to consider in metabolomics research are which biochemical fluid one intends to collect, whether the materials in the collection process up to sample preparation are suitable and do not interfere with the measurement method or the low-freezing storage facility.[75] Another important step is to consider at what temperatures the body fluid will be kept during the preparation process, as lower temperatures reduce the activity of cellular metabolism. It is generally considered that it is important to centrifuge samples as soon as possible, but consistency in time until centrifugation is even more important.[75] Therefore, a standardized protocol for the process of body fluids is essential. Other issues that are more obvious to be kept consistent are,

centrifugation time and force, and temperature of sample storage. For metabolomics and lipidomics, centrifugation (between 2300 and 4000 *g* for 5–10 min) of whole blood is recommended and for CSF 2000 *g* at 4°C to separate erythrocytes, leucocytes and platelets.[75] For serum samples, the coagulation process should be standardized (brand of tubes, kind of coagulation enhancer, clotting time and ambient temperature).[75, 80] On the other hand with stable molecules changes in sample handling do not have to be of large consequences, as has been shown when comparing different aspects (temperature, centrifugation and anti-enzymatic additives) of sample handling in amines in CSF.[81] When samples are kept for long-term storage, a storage temperature of -80°C or lower is advised.[75, 82] Regarding the patient, aspects of the diet, nutritional state as well as circadian rhythm can all affect the metabolome.[75, 83-86] Hence, one should try to keep these factors consistent during sample collection. It is also highly advised to keep track of a person's medications, smoking habits, daily intake of tea/coffee, and alcohol consumption.[75] Despite that these factors are crucial in the data quality of metabolomics research they are often not described in research papers.

Figure 1 Coupling of the different -omics, i.e. the genome, epigenome, transcriptome, proteome and metabolome



Genomic data can differ at different levels due to, for example, copy number variation (CNV), single nucleotide polymorphisms (SNPs) and mutations, at the genome level; at the epigenome level DNA methylation, histone modification and chromatin accessibility; gene expression and splicing at the transcriptome level; protein expression and post-translational modification at the proteome level; at the metabolome level the metabolic profile. Each variation in each level can be assessed with different techniques either by a targeted approach or an untargeted approach. Adapted from Ritchie et al.[79]

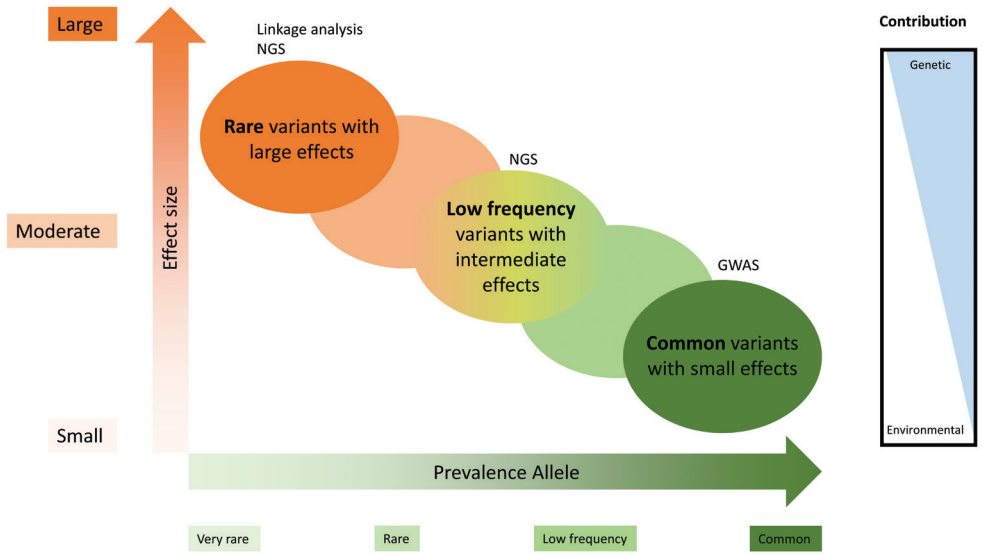
Rationale for genetic studies

When identifying genes involved in a disorder, different approaches are used depending on the disorder's architecture (i.e. monogenic, oligogenic or polygenic). The more oligogenic a disease is (i.e. the smaller the number of genes involved, with monogenic being the extreme), the larger the effect size of the associated gene variant(s) tends to be (**Figure 2**), in line with epidemiological data from disorders where rare disorders are monogenic and common disorders polygenic.

Hemiplegic migraine

Most of our knowledge of molecular mechanisms in migraine pathophysiology came from studying rare hemiplegic migraine (HM). The classical linkage method in migraine research was used to study large families with HM and this revealed a clear Mendelian (monogenic) type of inheritance. The approach led to the identification of three undisputed HM genes; *CACNA1A* (FHM1), *ATP1A2* (FHM2), and *SCN1A* (FHM3).[87-89]

Figure 2 Relationship between different types of hereditary (monogenic vs. polygenic) disorders.



Illustrating the relation to the allele frequency and the corresponding effect size as well as the contribution of genetic variants *vs.* environmental factors. Adapted from Manolio et al.[90]

In many patients with HM no pathogenic mutation has been detected in the HM genes.[91, 92] In recent years, whole-exome (next-generation) sequencing (WES) has been used to try and identify additional causal genes in patients without mutations in the known HM genes, but this has been proven difficult and no "fourth" gene has been identified thus far.[93] A study by Pelzer et al.[93] did,

however, show that patients with a more severe phenotype were more prone to have a causal mutation in one of the HM genes. Patients with a causal mutation in *CACNA1A*, *ATP1A2*, or *SCN1A* had a lower age-at-onset, more affected family members, and had attacks more frequently. Moreover, attacks were (i) brought about by mild head trauma, (ii) typically with extensive motor weakness, and (iii) with brainstem features, confusion, and brain oedema. Noteworthy, progressive ataxia and intellectual disability were only found in patients with a causal gene mutation.[93] As no mutation was found in "milder" patients, it was proposed that such HM patients may have the more extreme phenotype in the migraine with aura continuum.[91] Illustrative of this is a Finnish polygenic risk score study that showed that patients with HM, but without a high-penetrant disease-causing mutation in a known HM gene, carry an excess of genome-wide association studies (GWAS) variants associated with common migraine compared to patients suffering from the common migraine subtypes,[94] suggesting indeed a spectrum ranging from common low-risk variants to rare highly-penetrant mutations to contributing to the risk for migraine. Further support for this hypothesis are loss-of-function mutations in *PRRT2*, which do not cause HM on their own, but rather function as modifying genetic risk factors.[95] Illustrating the complex genetic architecture of HM is a recent whole-genome sequencing (WGS) where patients with HM were more likely to accumulate frameshift indels in multiple genes that have a role in synaptic signalling in the central nervous system compared to common migraine patients.[96]

Genetic studies in common migraine

Various twin and familial studies investigating the genetic and environmental susceptibility in migraine have shown that migraine is a multifactorial (complex) genetic disorder with a strong familial aggregation.[14, 15] The heritability of migraine was estimated to range from 35% to 60%.[97] Population-based studies have shown that the relative risk for a first-degree relative of a migraine patient is increased by 1.5- to 4-fold in comparison to a patient in the general population.[14] The risk was highest for those patients with a higher pain score and frequency of attacks, an early age of disease onset, and a migraine with aura phenotype.[14-16] Studies of twins identified a higher genetic load in migraine with aura compared with migraine without aura.[98] Migraine frequency, being the number of migraine days per month, appears mainly to be associated with a genetic predisposition in males.[16] A stronger family history of migraine is also associated with migraine with aura, a lower age-at-onset and more medication days.[16] For decades, identifying gene variants involved in complex disorders, such as migraine, has proven challenging.

Genetic studies in cluster headache

Twin and family studies have shown the involvement of genetic factors in cluster headache.[21] Notably, first-degree relatives have an increased relative risk between 5- and 18-fold, whereas second-degree relatives have a risk 1- to 3-fold higher than in the general population.[99] Thus far, most genetic studies have interrogated a limited number of variants in genes linked to presumed pathways in cluster headache.[100, 101] Variants in the *HCRTR2* gene were predominantly studied.

The *HCRTR2* gene encodes the G-protein coupled receptor hypocretin type 2 receptor that binds neuropeptides hypocretin-1 and -2 in the central nervous system. Such causal role of hypocretins makes sense as they have been implicated in sleep and arousal as well as pain modulation,[102] and levels were reported to be lower in CSF of patients with cluster headache.[103] However, initially positive genetic findings for *HCRTR2* associations[104-106] were not replicated in better-powered studies.[101, 107] Genes involved in circadian rhythmicity have also been investigated, but no association could be found.[108]

Genome-wide association studies

As a result of the improvement in DNA technology and the advancement of cost-effective genotyping platforms GWAS has become the method of choice to identify gene variants in complex traits in an untargeted approach in the last decade. Typically, in GWAS, several millions of single nucleotide polymorphisms (SNPs) are tested for association with a disorder by assessing differences in allele frequencies between large numbers of patients and controls. Of note, only common variants with a low to high minor allele frequency (≥0.01) are interrogated.

Since 2010, the International Headache Genetics Consortium (IHGC; www.headachegenetics.org/) has conducted several migraine GWAS, and with the increasing sample sizes, the number of associated gene variants steadily expanded. For cluster headache the first GWAS was performed in a very small, Italian study investigating patients with cluster headache.[109] They found a suggestive association with genetic variants in *ADCYAP1R1* and *MME*,[109] but the findings were not be replicated in a larger Swedish sample.[110] The hope is that larger GWAS will yield variants robustly associated with cluster headache.

Next-generation sequencing

A large part of the genetic variance and heritability in common diseases cannot be explained (usually referred to as "missing heritability") with a GWAS approach alone. One reason is that rarer variants (MAF<0.01), potentially with higher effect sizes, are not well interrogated by genotyping arrays typically utilised in the GWAS approach. Such mediate-effect-size variants can be identified using a next-generation sequencing (NGS) approach, i.e. by the simultaneous large-scale sequencing of the coding exons (whole-exome sequencing; WES) or the entire genome (whole-genome sequencing; WGS). In addition, the simultaneous sequencing of RNA transcripts ("transcriptome"; RNA-seq), either of bulk tissue or of its single nuclei can shed light on molecular mechanisms.

Only a few NGS studies have been performed in migraine thus far. Until now, WES was typically applied to cohorts of patients with HM, testing several hundred cases in an attempt to either find causal mutations in known HM genes or novel HM genes in patients that are negative for mutations in *CACNA1A*, *ATP1A2*, and *SCN1A*. Until now results have not led to additional

(undisputed) HM genes.[91-93,111,112] This may indicate that HM in mutation-negative patients may be oligogenic or polygenic, in line with the excess presence of common variants in such patients.[94] For cluster headache no gene sequencing studies have ever been performed which is logical as the gene array studies for this disease only just started.

An alternative approach to understanding molecular mechanisms involved in the pathophysiology of headache disorders is to study gene expression profiles. Contrary to genetic variation, gene expression is not fixed through life and expression is driven by both genetic and environmental factors.[113] Typically, an RNA-seq approach (i.e. simultaneous sequencing of coding (messenger) and non-coding RNAs in a sample) is for instance used to identify differences in expression between individuals with and without disease or over the course of an attack. Various RNA-seq studies have been performed in migraine, but the results are not unambiguous not in the least because of potential caveats of using peripheral blood, the main source of biomaterial for such studies in the case of migraine.[114] Gene expression studies in cluster headache are scarce. One study suggested the involvement of several brain-related mechanisms (voltage-gated channels and GABA receptor function), mitochondria, inflammation and intracellular signalling cascades.[115] Another study found an indication for inflammatory activity in the active phase of the disease.[116]

Further genetic studies

GWASs have proven successful in identifying many dozens of low-effect risk DNA variants for the more common forms of migraine with the number of associated DNA variants increasing steadily with larger sample sizes. Currently, next-generation sequencing, utilising whole-exome and -genome sequencing data, and other -omics data are being used to facilitate their functional interpretation and the discovery of additional risk factors. Various methods and analysis tools, such as genetic correlation, polygenic risk scores (PRSs) and causality analysis, are used to further characterise genetic risk factors.

Downstream bioinformatics methods

One way of making better use of the large number of small effect variants identified in migraine GWAS to have clinical benefit is the calculation of PRSs. A PRS is the combined effect of many common risk variants of genetic load for the discovery trait that can be used to estimate risk for a certain trait/phenotype in individuals in a target sample.[117] This is done by testing whether a higher PRS based on the discovery sample is associated with case status or a specific trait in the target sample via regression models. A PRS provides a promising possibility to investigate the shared genetic architecture between migraine with known and hitherto unknown co-morbidities or traits. The aggregation of migraine in families and the earlier age of onset of migraine can to some extent be contributed to common polygenic variations, where the PRS explained a larger part of the phenotype variance in familial cases, especially those with migraine with aura and hemiplegic migraine compared to population cases.[94]

Another way in which GWAS data can be used is by investigating the genetic relationships between traits, one of these analysis is Mendelian randomisation (MR). MR is able to entangle the pleiotropy that exists across many traits. In an MR analysis, genetic variants associated with an exposure are identified and regressed upon an outcome measurement to infer causality (*i.e.*, direction) of the association. Given the random assortment of alleles at gametogenesis in early life, this method is less likely to suffer from issues of confounding and reverse causation than methods used in conventional observational epidemiological studies.[118] For a successful MR analysis, three assumptions need to be fulfilled.[119] (I) Variants used as instrumental variables (IVs) need to be associated with the exposure. (II) The IVs only affect the outcome through the exposure, not through any other causal pathway. Factors that may lead to violation of this assumption include population stratification, LD and horizontal pleiotropy, the latter means that there is an (in)direct independent association of the IV (or another SNP in LD with the IV) with another trait that is not in the causal pathway of the investigated relation. (III) The IVs must not be associated with confounders. In one-directional MR the possible causal relation between trait X on trait Y is investigated, in bidirectional MR studies the directional effect from trait Y on trait X is also investigated.

Outline of this thesis

The research conducted for this thesis is divided in two parts. **Part 1** of the thesis focuses on biochemical studies in migraine. Here the biochemistry of migraine is investigated in: (i) a targeted approach, focusing on one or more related selected pathways of interest or, (ii) an untargeted approach aiming to simultaneously measure as many metabolites as possible from a biological sample. **Part 2** of the thesis focusses on genetical studies in both migraine and cluster headache using next-generation sequencing data and array genotyping data.

Part I Biochemistry of migraine

In **Chapter 2** we investigated whether the overall metabolic profile in blood of patients with migraine differed from those without migraine. Close to 100 metabolites were measured with ^{1}H-NMR spectroscopy in blood serum of 289 individuals with migraine and 1,360 individuals without migraine, all derived from a genetic isolate in the South-West of the Netherlands. **Chapter 3** describes whether CSF levels of amines, measured using an untargeted approach correlate with blood plasma levels in healthy volunteers. The study was then extrapolated to migraine patients. This chapter illustrates to what extent amine levels of CSF and blood relate to each other and seems to emphasize the role of blood-brain-barrier transport. **Chapter 4** investigates whether the endocannabinoid system is disrupted in interictal patients with migraine. To this end, the levels of three endocannabinoids in CSF were investigated in interictal (e.g. outside an attack) individuals with migraine with aura (n = 97) and without aura (n = 97) compared to healthy volunteers (n =

94). Endocannabinoids were measured using a previously validated micro-liquid chromatography-tandem mass spectrometry (micro-LC-MS/MS) technique. In **Chapter 5** the role of PGE_2 in the (early) phase of an induced migraine attack was investigated. To this end, PGE_2 plasma levels were measured towards in the (pre)ictal state of a glyceryl trinitrate (GTN) provoked attack in women with and without migraine.

Part II Genetics of different headache forms

In **Chapter 6** a GWAS in cluster headache is described. This study aims to demonstrate whether there are robust genetic associations for cluster headache. The study investigated 840 Dutch patients and a replication was performed in 144 Norwegian patients. In **Chapter 7** a meta-analysis of multiple GWAS studies of cluster headache with patients from Norway, Sweden, UK Germany, Denmark, Greece, Spain, Italy and the Netherlands (in total 4,043 patients) is conducted to not only confirm previous risk loci but also identify new disease risk loci. Using a Mendelian randomization approach it is investigated whether the intensity of cigarette smoking has a causal effect on cluster headache. **Chapter 8** describes a meta-analysis of multiple GWAS studies in migraine (102,084 migraine cases and 771,257 controls). Specific risk loci for migraine subtypes are investigated in a clinical sample. The aim of **Chapter 9** is to investigate whether there is an increased burden in hemiplegic migraine of missense variants in *CACNA1X* genes in patients without a high-penetrant disease-causing mutation in one of the well-known hemiplegic migraine genes (*CACNA1A*, *ATP1A2*, and *SCN1A*). The study illustrates the genetic complexity of hemiplegic migraine and the possibility of a spectrum ranging from high-risk rare mutations to low-risk common variants contributing to the risk for all forms of migraine.

Finally, **Chapter 10** provides a general discussion of the thesis together with suggestions for future research.

References

1. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. *Cephalalgia*. 2018;38(1):1-211.
2. Ferrari MD, Klever RR, Terwindt GM, Ayata C, van den Maagdenberg AM. Migraine pathophysiology: lessons from mouse models and human genetics. *Lancet Neurol*. 2015;14(1):65-80.
3. May A, Schwedt TJ, Magis D, et al. Cluster headache. *Nat Rev Dis Primers*. 2018;4:18006.
4. Ashina M, Terwindt GM, Al-Karagholi MA, et al. Migraine: disease characterisation, biomarkers, and precision medicine. *Lancet*. 2021;397(10283):1496-1504.
5. Ayata C. Cortical spreading depression triggers migraine attack: pro. *Headache*. 2010;50(4):725-730.
6. Charles A. Does cortical spreading depression initiate a migraine attack? Maybe not. *Headache*. 2010;50(4):731-733.
7. Eikermann-Haerter K, Ayata C. Cortical spreading depression and migraine. *Curr Neurol Neurosci Rep*. 2010;10(3):167-173.
8. Pietrobon D, Striessnig J. Neurobiology of migraine. *Nat Rev Neurosci*. 2003;4(5):386-398.
9. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition. *Cephalalgia*. 2018;38(1):1-211.
10. Dodick DW. A Phase-by-Phase Review of Migraine Pathophysiology. *Headache*. 2018;58 Suppl 1:4-16.
11. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology*. 1999;53(3):537-542.
12. Louter MA, Pelzer N, de Boer I, et al. Prevalence of lifetime depression in a large hemiplegic migraine cohort. *Neurology*. 2016;87(22):2370-2374.
13. Minen MT, Begasse De Dhaem O, Kroon Van Diest A, et al. Migraine and its psychiatric comorbidities. *J Neurol Neurosurg Psychiatry*. 2016;87(7):741-749.
14. Russell MB, Olesen J. Increased familial risk and evidence of genetic factor in migraine. *BMJ*. 1995;311(7004):541-544.
15. Stewart WF, Bigal ME, Kolodner K, et al. Familial risk of migraine: variation by proband age at onset and headache severity. *Neurology*. 2006;66(3):344-348.
16. Pelzer N, Louter MA, van Zwet EW, et al. Linking migraine frequency with family history of migraine. *Cephalalgia*. 2018:333102418783295.
17. Nesbitt AD, Goadsby PJ. Cluster headache. *BMJ*. 2012;344:e2407.
18. Frederiksen HH, Lund NL, Barloese MC, Petersen AS, Jensen RH. Diagnostic delay of cluster headache: A cohort study from the Danish Cluster Headache Survey. *Cephalalgia*. 2020;40(1):49-56.
19. Fischera M, Marziniak M, Gralow I, Evers S. The incidence and prevalence of cluster headache: a meta-analysis of population-based studies. *Cephalalgia*. 2008;28(6):614-618.
20. Lund N, Petersen A, Snoer A, Jensen RH, Barloese M. Cluster headache is associated with unhealthy lifestyle and lifestyle-related comorbid diseases: Results from the Danish Cluster Headache Survey. *Cephalalgia*. 2019;39(2):254-263.
21. Sjaastad O, Shen JM, Stovner LJ, Elsas T. Cluster headache in identical twins. *Headache*. 1993;33(4):214-217.
22. Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. *Brain*. 1994;117 (Pt 1):199-210.
23. Leao aap. spreading depression of activity in the cerebral cortex. *J Neurophysiol*. 1944;7(6):359-390.
24. Ferrari MDP, Klever RRM, Terwindt GMMD, Ayata CMD, van den Maagdenberg AMJMP. Migraine pathophysiology: lessons from mouse models and human genetics. *Lancet Neurology, The*. 2015;14(1):65-80.

25. Goadsby PJ, Holland PR, Martins-Oliveira M, et al. Pathophysiology of Migraine: A Disorder of Sensory Processing. *Physiol Rev*. 2017;97(2):553-622.
26. Zhang X, Levy D, Kainz V, et al. Activation of central trigeminovascular neurons by cortical spreading depression. *Ann Neurol*. 2011;69(5):855-865.
27. Hadjikhani N, Sanchez Del Rio M, Wu O, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc Natl Acad Sci U S A*. 2001;98(8):4687-4692.
28. Charles AC, Baca SM. Cortical spreading depression and migraine. *Nat Rev Neurol*. 2013;9(11):637-644.
29. Bernstein C, Burstein R. Sensitization of the trigeminovascular pathway: perspective and implications to migraine pathophysiology. *J Clin Neurol*. 2012;8(2):89-99.
30. Pietrobon D. Ion channels in migraine disorders. *Current Opinion in Physiology*. 2018;2:98-108.
31. Davis RJ, Murdoch CE, Ali M, et al. EP4 prostanoid receptor-mediated vasodilatation of human middle cerebral arteries. *Br J Pharmacol*. 2004;141(4):580-585.
32. Yokota C, Inoue H, Kuge Y, et al. Cyclooxygenase-2 expression associated with spreading depression in a primate model. *J Cereb Blood Flow Metab*. 2003;23(4):395-398.
33. Maniyar FH, Sprenger T, Monteith T, Schankin C, Goadsby PJ. Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks. *Brain*. 2014;137(Pt 1):232-241.
34. Hoffmann J, May A. Diagnosis, pathophysiology, and management of cluster headache. *The Lancet Neurology*. 2018;17(1):75-83.
35. Wei DY, Goadsby PJ. Cluster headache pathophysiology - insights from current and emerging treatments. *Nat Rev Neurol*. 2021;17(5):308-324.
36. May A, Goadsby PJ. The trigeminovascular system in humans: pathophysiologic implications for primary headache syndromes of the neural influences on the cerebral circulation. *J Cereb Blood Flow Metab*. 1999;19(2):115-127.
37. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature*. 2005;437(7063):1257-1263.
38. May A, Bahra A, Büchel C, Frackowiak RS, Goadsby PJ. Hypothalamic activation in cluster headache attacks. *Lancet*. 1998;352(9124):275-278.
39. Chan D, Ng LL. Biomarkers in acute myocardial infarction. *BMC Med*. 2010;8:34.
40. Molina R, Escudero JM, Augé JM, et al. HE4 a novel tumour marker for ovarian cancer: comparison with CA 125 and ROMA algorithm in patients with gynaecological diseases. *Tumour Biol*. 2011;32(6):1087-1095.
41. Høgdall C, Fung ET, Christensen IJ, et al. A novel proteomic biomarker panel as a diagnostic tool for patients with ovarian cancer. *Gynecol Oncol*. 2011;123(2):308-313.
42. Olesen J, Iversen HK, Thomsen LL. Nitric oxide supersensitivity: a possible molecular mechanism of migraine pain. *Neuroreport*. 1993;4(8):1027-1030.
43. Thomsen LL, Kruuse C, Iversen HK, Olesen J. A nitric oxide donor (nitroglycerin) triggers genuine migraine attacks. *Eur J Neurol*. 1994;1(1):73-80.
44. Antonova M, Wienecke T, Olesen J, Ashina M. Prostaglandin E(2) induces immediate migraine-like attack in migraine patients without aura. *Cephalalgia*. 2012;32(11):822-833.
45. Wienecke T, Olesen J, Ashina M. Prostaglandin I2 (epoprostenol) triggers migraine-like attacks in migraineurs. *Cephalalgia*. 2010;30(2):179-190.
46. Ashina M, Hansen JM, Olesen J. Pearls and pitfalls in human pharmacological models of migraine: 30 years' experience. *Cephalalgia*. 2013;33(8):540-553.
47. Hansen JM, Hauge AW, Olesen J, Ashina M. Calcitonin gene-related peptide triggers migraine-like attacks in patients with migraine with aura. *Cephalalgia*. 2010;30(10):1179-1186.
48. Bogucki A. Studies on nitroglycerin and histamine provoked cluster headache attacks. *Cephalalgia*. 1990;10(2):71-75.

49. Ferrari MD, Odink J, Tapparelli C, et al. Serotonin metabolism in migraine. *Neurology*. 1989;39(9):1239-1242.
50. Onderwater GLJ, van Dongen RM, Harms AC, et al. Cerebrospinal fluid and plasma amine profiles in interictal migraine. *Ann Neurol*. 2022; 93(4):715-728
51. 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*. 2000;86(1-2):133-138.
52. Cernuda-Morollon E, Larrosa D, Ramon C, et al. Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine. *Neurology*. 2013;81(14):1191-1196.
53. Fan PC, Kuo PH, Chang SH, et al. Plasma calcitonin gene-related peptide in diagnosing and predicting paediatric migraine. *Cephalalgia*. 2009;29(8):883-890.
54. Fekrazad R, Sardarian A, Azma K, et al. Interictal levels of calcitonin gene related peptide in gingival crevicular fluid of chronic migraine patients. *Neurol Sci*. 2018;39(7):1217-1223.
55. Fusayasu E, Kowa H, Takeshima T, Nakaso K, Nakashima K. Increased plasma substance P and CGRP levels, and high ACE activity in migraineurs during headache-free periods. *Pain*. 2007;128(3):209-214.
56. Jang MU, Park JW, Kho HS, Chung SC, Chung JW. Plasma and saliva levels of nerve growth factor and neuropeptides in chronic migraine patients. *Oral Dis*. 2011;17(2):187-193.
57. Rodriguez-Osorio X, Sobrino T, Brea D, et al. Endothelial progenitor cells: a new key for endothelial dysfunction in migraine. *Neurology*. 2012;79(5):474-479.
58. Gallai V, Sarchielli P, Floridi A, et al. Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. *Cephalalgia*. 1995;15(5):384-390.
59. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol*. 1990;28(2):183-187.
60. Juhasz G, Zsombok T, Modos EA, et al. NO-induced migraine attack: strong increase in plasma calcitonin gene-related peptide (CGRP) concentration and negative correlation with platelet serotonin release. *Pain*. 2003;106(3):461-470.
61. Sarchielli P, Alberti A, Codini M, Floridi A, Gallai V. Nitric oxide metabolites, prostaglandins and trigeminal vasoactive peptides in internal jugular vein blood during spontaneous migraine attacks. *Cephalalgia*. 2000;20(10):907-918.
62. Gupta R, Ahmed T, Banerjee B, Bhatia M. Plasma calcitonin gene-related peptide concentration is comparable to control group among migraineurs and tension type headache subjects during inter-ictal period. *J Headache Pain*. 2009;10(3):161-166.
63. Lee MJ, Lee SY, Cho S, Kang ES, Chung CS. Feasibility of serum CGRP measurement as a biomarker of chronic migraine: a critical reappraisal. *J Headache Pain*. 2018;19(1):53.
64. Oterino A, Toriello M, Palacio E, et al. Analysis of endothelial precursor cells in chronic migraine: a case-control study. *Cephalalgia*. 2013;33(4):236-244.
65. Tvedskov JF, Lipka K, Ashina M, et al. No increase of calcitonin gene-related peptide in jugular blood during migraine. *Ann Neurol*. 2005;58(4):561-568.
66. Diener H-C. CGRP antibodies for migraine prevention — new kids on the block. *Nature Reviews Neurology*. 2019;15(3):129-130.
67. Edvinsson L, Haanes KA, Warfvinge K, Krause DN. CGRP as the target of new migraine therapies - successful translation from bench to clinic. *Nat Rev Neurol*. 2018;14(6):338-350.
68. Goadsby PJ, Edvinsson L. Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. *Brain*. 1994;117 (Pt 3):427-434.
69. Snoer A, Vollesen ALH, Beske RP, et al. Calcitonin-gene related peptide and disease activity in cluster headache. *Cephalalgia*. 2019;39(5):575-584.

70. Fanciullacci M, Alessandri M, Figini M, Geppetti P, Michelacci S. Increase in plasma calcitonin gene-related peptide from the extracerebral circulation during nitroglycerin-induced cluster headache attack. *Pain*. 1995;60(2):119-123.

71. Ashina H, Schytz HW, Ashina M. CGRP in human models of primary headaches. *Cephalalgia*. 2016;38(2):353-360.

72. Messlinger K, Vogler B, Kuhn A, et al. CGRP measurements in human plasma - a methodological study. *Cephalalgia*. 2021;41(13):1359-1373.

73. Kuehnbaum NL, Britz-McKibbin P. New advances in separation science for metabolomics: resolving chemical diversity in a post-genomic era. *Chem Rev*. 2013;113(4):2437-2468.

74. Fiehn O, Robertson D, Griffin J, et al. The metabolomics standards initiative (MSI). *Metabolomics*. 2007;3(3):175-178.

75. Lehmann R. From bedside to bench-practical considerations to avoid pre-analytical pitfalls and assess sample quality for high-resolution metabolomics and lipidomics analyses of body fluids. *Anal Bioanal Chem*. 2021;413(22):5567-5585.

76. Carraro P, Zago T, Plebani M. Exploring the initial steps of the testing process: frequency and nature of pre-preanalytic errors. *Clin Chem*. 2012;58(3):638-642.

77. Lippi G, Guidi GC, Mattiuzzi C, Plebani M. Preanalytical variability: the dark side of the moon in laboratory testing. *Clin Chem Lab Med*. 2006;44(4):358-365.

78. Szecsi PB, Ødum L. Error tracking in a clinical biochemistry laboratory. *Clin Chem Lab Med*. 2009;47(10):1253-1257.

79. Ritchie MD, Holzinger ER, Li R, Pendergrass SA, Kim D. Methods of integrating data to uncover genotype–phenotype interactions. *Nature Reviews Genetics*. 2015;16(2):85-97.

80. Liu X, Hoene M, Wang X, et al. Serum or plasma, what is the difference? Investigations to facilitate the sample material selection decision making process for metabolomics studies and beyond. *Anal Chim Acta*. 2018;1037:293-300.

81. Noga MJ, Zielman R, van Dongen RM, et al. Strategies to assess and optimize stability of endogenous amines during cerebrospinal fluid sampling. *Metabolomics*. 2018;14(4):44.

82. Teunissen CE, Petzold A, Bennett JL, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. *Neurology*. 2009;73(22):1914-1922.

83. Bar N, Korem T, Weissbrod O, et al. A reference map of potential determinants for the human serum metabolome. *Nature*. 2020;588(7836):135-140.

84. Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. *Proc Natl Acad Sci U S A*. 2012;109(7):2625-2629.

85. Kasukawa T, Sugimoto M, Hida A, et al. Human blood metabolite timetable indicates internal body time. *Proc Natl Acad Sci U S A*. 2012;109(37):15036-15041.

86. Chua EC, Shui G, Lee IT, et al. Extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans. *Proc Natl Acad Sci U S A*. 2013;110(35):14468-14473.

87. Ophoff RA, Terwindt GM, Vergouwe MN, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell*. 1996;87(3):543-552.

88. Dichgans M, Freilinger T, Eckstein G, et al. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet*. 2005;366(9483):371-377.

89. De Fusco M, Marconi R, Silvestri L, et al. Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat Genet*. 2003;33(2):192-196.

90. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature*. 2009;461(7265):747-753.

91. Hiekkala ME, Vuola P, Artto V, et al. The contribution of CACNA1A, ATP1A2 and SCN1A mutations in hemiplegic migraine: A clinical and genetic study in Finnish migraine families. *Cephalalgia*. 2018;38(12):1849-1863.

92. Sutherland HG, Maksemous N, Albury CL, et al. Comprehensive Exonic Sequencing of Hemiplegic Migraine-Related Genes in a Cohort of Suspected Probands Identifies Known and Potential Pathogenic Variants. *Cells*. 2020;9(11) :2368.

93. Pelzer N, Haan J, Stam AH, et al. Clinical spectrum of hemiplegic migraine and chances of finding a pathogenic mutation. *Neurology*. 2018;90(7):e575-e582.

94. Gormley P, Kurki MI, Hiekkala ME, et al. Common Variant Burden Contributes to the Familial Aggregation of Migraine in 1,589 Families. *Neuron*. 2018;99(5):1098.

95. Pelzer N, de Vries B, Kamphorst JT, et al. PRRT2 and hemiplegic migraine: a complex association. *Neurology*. 2014;83(3):288-290.

96. Rasmussen AH, Olofsson I, Chalmer MA, Olesen J, Hansen TF. Higher burden of rare frameshift indels in genes related to synaptic transmission separate familial hemiplegic migraine from common types of migraine. *J Med Genet*. 2020;57(9):610-616.

97. Polderman TJ, Benyamin B, de Leeuw CA, et al. Meta-analysis of the heritability of human traits based on fifty years of twin studies. *Nat Genet*. 2015;47(7):702-709.

98. Ulrich V, Gervil M, Kyvik KO, Olesen J, Russell MB. Evidence of a genetic factor in migraine with aura: a population-based Danish twin study. *Ann Neurol*. 1999;45(2):242-246.

99. Russell MB. Epidemiology and genetics of cluster headache. *Lancet Neurol*. 2004;3(5):279-283.

100. May A, Bahra A, Buchel C, Frackowiak RS, Goadsby PJ. Hypothalamic activation in cluster headache attacks. *Lancet*. 1998;352(9124):275-278.

101. Gibson KF, Santos AD, Lund N, Jensen R, Stylianou IM. Genetics of cluster headache. *Cephalalgia*. 2019;39(10):1298-1312.

102. Razavi BM, Hosseinzadeh H. A review of the role of orexin system in pain modulation. *Biomed Pharmacother*. 2017;90:187-193.

103. Barloese M, Jennum P, Lund N, et al. Reduced CSF hypocretin-1 levels are associated with cluster headache. *Cephalalgia*. 2015;35(10):869-876.

104. Rainero I, Gallone S, Valfrè W, et al. A polymorphism of the hypocretin receptor 2 gene is associated with cluster headache. *Neurology*. 2004;63(7):1286-1288.

105. Fourier C, Ran C, Steinberg A, et al. Analysis of HCRTR2 Gene Variants and Cluster Headache in Sweden. *Headache*. 2019;59(3):410-417.

106. Schürks M, Kurth T, Geissler I, et al. Cluster headache is associated with the G1246A polymorphism in the hypocretin receptor 2 gene. *Neurology*. 2006;66(12):1917-1919.

107. Weller CM, Wilbrink LA, Houwing-Duistermaat JJ, et al. Cluster headache and the hypocretin receptor 2 reconsidered: a genetic association study and meta-analysis. *Cephalalgia*. 2015;35(9):741-747.

108. Ofte HK, Tronvik E, Alstadhaug KB. Lack of association between cluster headache and PER3 clock gene polymorphism. *J Headache Pain*. 2015;17:18.

109. Bacchelli E, Cainazzo MM, Cameli C, et al. A genome-wide analysis in cluster headache points to neprilysin and PACAP receptor gene variants. *J Headache Pain*. 2016;17(1):114.

110. Ran C, Fourier C, Michalska JM, et al. Screening of genetic variants in ADCYAP1R1, MME and 14q21 in a Swedish cluster headache cohort. *J Headache Pain*. 2017;18(1):88.

111. Ibrahim O, Sutherland HG, Maksemous N, et al. Exploring Neuronal Vulnerability to Head Trauma Using a Whole Exome Approach. *J Neurotrauma*. 2020;37(17):1870-1879.

112. Gil-Perotín S, Jaijo T, Verdú AG, et al. Epilepsy, status epilepticus, and hemiplegic migraine coexisting with a novel SLC4A4 mutation. *Neurol Sci*. 2021;42(9):3647-3654.

113. Bar-Joseph Z, Gitter A, Simon I. Studying and modelling dynamic biological processes using time-series gene expression data. *Nature reviews Genetics*. 2012;13(8):552-564.

114. Gerring Z, Rodriguez-Acevedo AJ, Powell JE, et al. Blood gene expression studies in migraine: Potential and caveats. *Cephalalgia*. 2016;36(7):669-678.

115. Eising E, Pelzer N, Vijfhuizen LS, et al. Identifying a gene expression signature of cluster headache in blood. *Sci Rep*. 2017;7:40218.

116. Sjöstrand C, Duvefelt K, Steinberg A, et al. Gene expression profiling in cluster headache: a pilot microarray study. *Headache*. 2006;46(10):1518-1534.

117. Purcell SM, Wray NR, Stone JL, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460(7256):748-752.

118. Zheng J, Baird D, Borges MC, et al. Recent Developments in Mendelian Randomization Studies. *Current epidemiology reports*. 2017;4(4):330-345.

119. Haycock PC, Burgess S, Wade KH, et al. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr*. 2016;103(4):965-978.