Unraveling the genetic architecture of migraine: exploring the vascular components

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CHAPTER 1

General introduction

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General introduction

Clinical characteristics of migraine

Migraine is a common, debilitating neurovascular disorder with a life-time prevalence of 15-30% worldwide.[1-3] Migraine manifests as recurrent attacks of headache with a range of accompanying symptoms (Table 1). There are two main migraine types depending on the absence/presence of an aura: migraine without aura (MO) and migraine with aura (MA).[4] Auras occur in approximately one-third of migraine patients and are characterized by transient focal neurologic symptoms (i.e., visual, sensory, motor or speech disturbances) that display positive phenomena and a succession or expansion of symptoms.[3-5] In migraine with aura, the headache often also occurs, but the aura phase may also occur without subsequent migrainous headache. Another relevant subtyping of migraine is episodic *vs.* chronic migraine, which is based on number of monthly migraine and headache days. Patients have chronic migraine when they have headache on at least 15 days per month, with eight of these being migraine days, for at least three months.[4]

Table 1. Criteria for migraine with and without aura in accordance with the International Classification of headache disorders (ICHD), third edition.[4]

ICHD-3 criteria for migraine
1.1 Migraine without aura
A. At least five attacks fulfilling criteria B–D
B. Headache attacks lasting 4–72 h (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, vomiting, or both
2. Photophobia and phonophobia
E. Not better accounted for by another ICHD-3 diagnosis
1.2 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 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 min
2. Two or more aura symptoms occur in succession
3. Each individual aura symptom lasts 5–60 min
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 min, by headache
D. Not better accounted for by another ICHD-3 diagnosis

The diverse and changing nature of migraine challenges adequate comparison of migraine types. Migraine type as well as migraine frequency can change over time.[6-8] This means that attack type and attack frequency are not stationary, but rather dynamic traits. This may especially be problematic when studying migraine pathophysiology. In most large-scale studies, patients are often labeled with a life-time diagnosis, e.g., if a patient had migraine with aura in their twenties, they are still considered migraine with aura patients even if they only suffer from migraine without aura attacks from age 40.

Pathophysiology of migraine

The pathogenesis of the migraine headache involves activation of the trigeminovascular system, which includes the trigeminal ganglion and its peripheral axonal projections that innervate pain-sensitive intracranial structures such as the meninges (Fig. 1).[9] Additionally, axonal projections arise from trigeminal ganglion cells and convey nociceptive impulses to second-order neurons in the brainstem.

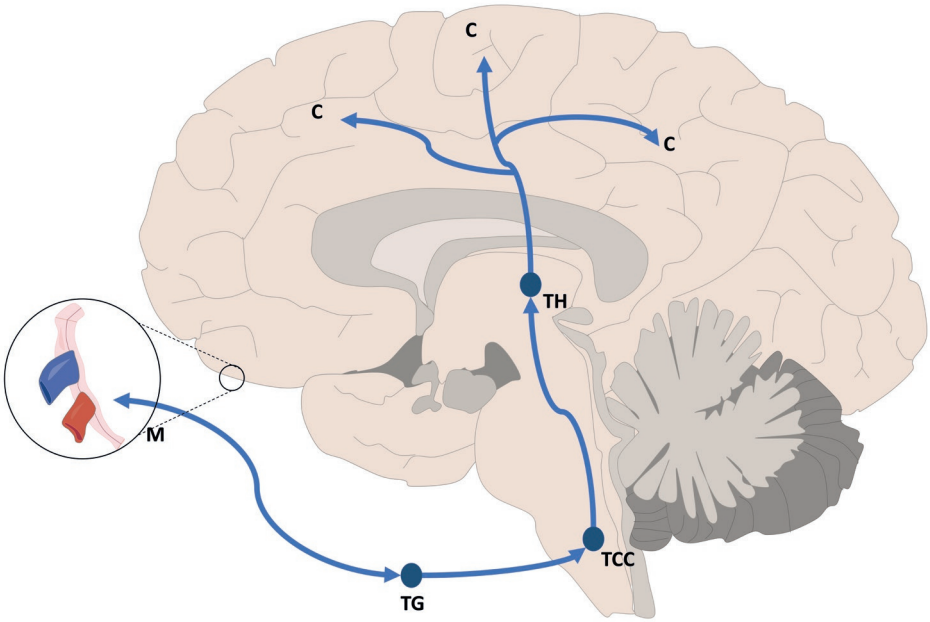

Figure 1. Pathophysiology of migraine.

Peripheral axonal projections of the trigeminal ganglion (TG) innervate pain-sensitive intracranial structures such as the meninges (M). Trigeminal afferents arising from the TG transmit sensory input towards the trigeminocervical complex (TCC). The TCC coordinates trigeminal pain processing between the peripheral and central nervous system. Projecting neurons travel via the trigeminothalamic tracts and synapse on multiple thalamic nuclei (TH). En route collateral projections target several additional nuclei (among which are the locus coeruleus, the periaqueductal gray and the hypothalamus). Thalamocortical neurons convey the sensory input to multiple cortical regions (C).

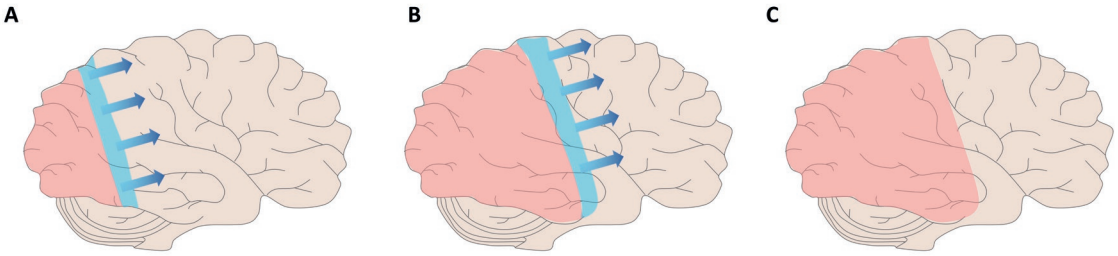

Figure 2. A schematic representation of cortical spreading depolarization.
Cortical spreading depolarization (CSD) is a brief wave of neuronal and glial depolarization that slowly self-propagates across the cerebral cortex (blue) (A and B), followed by long-lasting suppression of brain activity (pink) (A-C).

These neurons, in turn, project to third-order neurons in the thalamus, which then convey nociceptive impulses to a wide array of cortical areas that are involved in pain processing, including the somatosensory cortex (Fig. 1).[9]

Migraine aura is believed to be caused by cortical spreading depolarization (CSD) (Fig. 2). CSD is a wave of electrophysiological hyperactivity followed by a wave of inhibition. Spreading depolarization describes a phenomenon characterized by intense neuronal and glial depolarization that classically starts in the occipital (visual) cortex and slowly (~3 mm/min) propagates frontally through the cortex, until it encounters a less sensitive part of the brain and stops or redirects, thus explaining the wide variety of aura patterns patients report.[10-13] After the initial wave, affected neurons can be depolarized for several minutes up to an hour. Therefore, originally the term 'cortical spreading depression' was used although the term depolarization is now preferred. Genetic factors may lead to a lower CSD threshold and could thereby increase migraine susceptibility.[14-16]

Genetics of migraine

It has long been established that there is a strong genetic component in migraine.[17,18] This is exemplified by the fact that there are several monogenic migraine syndromes in which migraine is either the main symptom or part of a complex of symptoms. With respect to common forms of migraine (i.e., migraine with and without aura), large genome-wide association studies (GWAS) have greatly expanded our knowledge of the genetic variants involved, emphasizing the role of both neuronal and vascular pathways.[19] Examining the genetic structure of prevalent migraine types enhanced

our understanding of the connections among distinct (sub)types, as well as their associations with disorders coexisting with migraine in the same patient. Currently, the acquired knowledge has little utility in diagnosis or tailoring of treatments. This may change in the future with more advanced genetic studies focusing for instance on neuropharmacology (pharmacogenetics). In contrast, the clinical significance of recognizing monogenic migraine syndromes and understanding their underpinning genetic architecture has long been established. Recognizing monogenetic migraine patients is vital for accurate diagnosis, counseling, and treatment.

Complex genetics of common types of migraine

Common migraine is regarded as a complex inherited neurological disorder. Complex traits are typically caused by a combination of multiple genetic factors (each with a small effect size) and environmental factors.[20,21] In migraine family studies, heritability, defined as the contribution of genetic factors to susceptibility of a trait, was estimated to range from 35% up to 60%.[22]

Apart from genetic heterogeneity, the search for gene variants in the common forms of migraine is complicated by clinical heterogeneity. In this regard, it is crucial to note that there are currently no reliable biomarkers for migraine. Therefore, it is impossible to establish a migraine diagnosis based on clear quantifiable (objective) factors. Of note, the current diagnostic criteria of the ICHD-3 [4] are useful to diagnose patients in a clinical setting but may be less suited for pathophysiological research, simply because multiple combinations of symptoms (e.g., pulsating headache, nausea, vomiting) can lead to the same end-diagnosis, but might not necessarily been brought about by the same molecular mechanism.

Genome-Wide Association Studies

Improved technology and the development of cost-effective genotyping platforms has enabled genome-wide association studies (GWAS). GWAS has become the method of choice, to identify gene variants in complex traits. As no prior hypotheses need to be formulated, GWAS can be used to *generate* hypotheses. Typically, in a GWAS, several hundred thousand to millions of single nucleotide polymorphisms (SNPs) are tested for association with a trait (e.g., migraine) by assessing differences in allele frequencies between large numbers (several thousand or even tens or hundred thousand) patients and controls. Of note, in a GWAS typically only variants with a moderate to high minor allele frequency (≥0.01) are genotyped. Therefore, rare variants are overlooked. GWAS is an ideal method when identifying multiple common variants with small effect sizes that play a role in a disorder. The downside of the method is, given the small effect sizes that are observed in a GWAS, the need

for an exceptionally large sample size to obtain a significant association. As such, the direct clinical relevance of these findings may be limited. Furthermore, the most commonly employed method to ascertain the causal gene defect based on the most significant SNP in a locus in GWAS is to identify the gene nearest to that SNP. This does not necessarily mean that the correct gene is picked for each locus. Fortunately, additional bioinformatics tools can be used to determine the correct gene with more validity.[23] The migraine GWAS published in 2016 included 59,674 migraine cases and 316,078 controls; 38 genomic regions associated with migraine were identified, of which 28 were previously unknown.[24]

From a clinical perspective, gene variants with low relative risks may at first sight seem of limited value, but their identification does shed light on possible novel mechanisms that are relevant to migraine pathophysiology. Thus, genetic research has the potential to discover many yet unknown migraine pathways. Insight in these pathways can contribute to the development of new treatments. It has been suggested that selecting genetically supported targets could double the success rate of drug development.[25]

Missing heritability of migraine

All identified migraine-associated SNPs show small effect sizes and thus account for a small part of migraine genetic heritability; when combining all currently known associated SNPs they add up to no more than 14.63% (95%CI = 13.79-15.47) heritability;[24] where the total contribution of genetic factors is estimated to be between 35% to 60% based on family studies.[22,26] This is a common problem in complex traits and is commonly known as the missing heritability problem, which indicates that genetic variants in GWAS may not explain all of the heritability in complex traits. Most likely rarer, medium- to high-risk, alleles that are not captured by a GWAS approach play an important role.[27] Finding an association for rarer alleles is, however, notoriously difficult, as it necessitates even larger cohorts to achieve a sufficiently sizable representation of these rare variants in order to attain statistical significance. Successes of shedding light on missing heritability for any trait are scarce or essentially missing,[28] possibly because the problem of missing heritability was initially ill-defined as it did not take structural variants, variants in the mitochondrial DNA, epigenetic mechanisms and gene–gene and gene-environment interactions into account.

Structural variation and mitochondrial DNA

Unlike SNPs, GWAS cannot assess the contribution of other genetic variations, especially not of structural variations. Copy number variation (CNV) occurs when sections of the genome are repeated and the number of repeats in the genome varies

between individuals. In addition to well-known sporadic chromosomal microdeletion syndromes and Mendelian diseases, many common complex traits can result from CNVs.[29] Within the migraine field this is not a well-researched area. One study has tested whether length variations in the second polymorphic CAG repeat in exon 1 of the *KCNN3* gene, are involved in susceptibility to MA and MO and found no association.[30] It is unclear if and to what extend these repeats or CNV in general have a role in the genetic basis of migraine.

Another part of the missing heritability can be explained by genetic variants in mitochondrial DNA (mtDNA). Mitochondrial DNA is separated from nuclear DNA and inherited exclusively along the maternal lineage. Each cell contains hundreds to thousands of copies of mtDNA, and there is genetic variation between mtDNA molecules.[31] Interestingly, migraine-like headaches have been reported in several mitochondrial disorders, such as mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS).[32] In addition, structurally abnormal mitochondria and findings indicative of impaired energy metabolism have been demonstrated in migraine.[33] While small candidate gene association studies focusing on mtDNA have reported associations,[34] these could not be replicated in a mitochondrial GWAS.[35] Furthermore, this mitochondrial GWAS found no additional associations with migraine. As only ~4000 cases were included, it seems likely that if mitochondrial DNA is part of the puzzle, a larger sample size is required to find such variants robustly associated with migraine.

Epigenetic Mechanisms

Another explanation for the missing heritability phenomenon can be that the heritability not only resides in the genome, but also in the epigenome. Epigenetics encompasses changes to the DNA structure without changing the genetic code. Epigenetic changes have been implicated in several neurologic disorders.[36] They can be dynamic, be passed on through cell divisions and even to offspring. The main epigenetic modifications are DNA methylation and post-translational modification of histone proteins. So far, small steps have been taken to dissect the epigenetic contribution towards migraine.[37] The first genome-wide analysis of DNA methylation in migraine identified 62 independent differentially methylated regions in blood samples without distinguishing between MO and MA.[38]

Gene-gene and gene-environment interactions

Gene-gene interaction (epistasis) is the modification of the effect of one gene on disease by another gene, or several others. Gene-environment interaction occurs when different genotypes respond to environmental variation in different ways.

This field of research is still in its infancy, also within migraine research.[39-41] It needs mentioning that the interactions are very challenging to study given the enormous possibilities of interactions and computational *force majeure* is needed to find them. Still, in other complex multifactorial diseases, such as multiple sclerosis and breast cancer, epistasis and gene-environment interactions have been suggested to be important in pathophysiology.[42-46]

Shared genetic architecture of migraine with other brain disorders

The comorbidity occurring between two disorders can be spurious due to selection bias or can reflect a unidirectional causal relationship (i.e., migraine causes (or is caused by) the comorbid disorder). It also may be due to shared genetic and/or environmental factors that underlie both migraine and the other disorder.[47] In other words, to certain extent, there may be a shared genetic architecture. It can be an attractive strategy for genetic studies, as it will reduce genetic heterogeneity by selecting those migraine patients that also suffer from the other disorder. There is limited sharing of genetic information *between* neurologic and psychiatric disorders.[48] There was but one exception, which is the observed correlation of migraine, a neurologic disorder, with depression, a psychiatric disorder.

Genetic correlation between Migraine and Psychiatric Disorders

The Brainstorm Consortium study showed that migraine was correlated to several psychiatric disorders, including major depressive disorder.[48] This is in agreement with a GWAS study specifically searching for a shared genetic background for depression and migraine. In that meta-analysis in the combined 8,045,569 SNPs of the migraine GWAS and the top 10,000 SNPs from a major depressive disorder GWAS, three novel risk loci were identified that were associated with both disorders.[49] Furthermore, gene-based association analyses revealed significant enrichment of genes associated with both migraine and major depressive disorder. Pathway analyses suggested several important pathways, especially neural-related pathways of signaling and ion channel regulation, to be involved in this shared etiology.[49] In addition, in a previous study in 2,652 participants of the Dutch Erasmus Rucphen Family Study, a genetic isolate, the contribution of shared genetic factors in migraine and depression was investigated by comparing heritability estimates for migraine with and without adjustment for symptoms of depression, and by comparing the heritability scores of depression between migraineurs and controls.[50] Comparison of the heritability scores for depression between patients with migraine and controls showed a genetic correlation between the Hospital Anxiety and Depression Scale-Depression (HADS-D) score and MA. This is in line with epidemiological studies demonstrating

a bidirectional association between depression and migraine.[51] Moreover, a genetic association was found between migraine and anxious depression.[52] This is especially interesting as there is a strong and consistent relationship between migraine and anxiety. The comorbidity of co-occurrence for migraine and anxiety has an average OR of 2.33 (95%CI: 2.20-2.47) among cross sectional studies and an average RR of 1.63 (95%CI: 1.37-1.93) for cohort studies.[53-55] There is limited evidence explaining this comorbidity. Nonetheless, the Brainstorm consortium did demonstrate a possible genetic association between migraine and anxiety disorders.[48]

Genetic correlation between migraine and neurologic disorders

Although the Brainstorm Consortium could not identify significant genetic risk overlap across neurologic disorders, their study suggested genetic sharing between epilepsy and migraine.[48] An explanation for the lack of statistical significance may be that a relatively small epilepsy cohort was used besides the fact that epilepsy represents more heterogenous disorder(s) with a wide variety of underling mechanisms. A genetic connection between migraine and epilepsy is anticipated, as patients with hemiplegic migraine frequently experience concurrent epileptic and (hemiplegic) migraine attacks, as demonstrated by previous research.[56] Additionally, epidemiological studies have revealed comorbidity between common migraine and epilepsy.[57] Of note, the Brainstorm Consortium failed to find a genetic correlation between ischemic stroke and migraine, despite an earlier study using GWAS data revealing substantial genetic overlap between both disorders,[58] and epidemiologic studies repeatedly demonstrating an increased risk of ischemic stroke in migraine patients.[59,60] Future studies with larger cohorts and more detailed clinical and genetic data may be able to further substantiate the genetic correlation for migraine and comorbid neurologic disorders, foremost of epilepsy and stroke.

Genetic correlation between migraine and 'headache'

A study from UK Biobank that had 74,461 cases and 149,312 controls identified 28 genomic loci associated with a broadly-defined headache phenotype.[61] Of note, only 14 of the found loci where previously identified in a migraine GWAS.[24] There are various possible explanations for this finding. These loci are involved in a broader headache pathway, as part of the cases may in fact be patients with tension type headache.[62] However, giving the robustness of the associated SNPs in the migraine GWAS and the fact that six of the loci identified in the headache GWAS were also identified in clinical-based migraine GWAS may suggest that part of the identified SNPs are not only migraine specific but may be related to headache symptoms in general.[24,63]

Monogenic migraine disorders

In rare monogenic migraine syndromes, a single DNA mutation is sufficient to cause disease. The identification of causal gene defects for such syndromes is more straightforward than for complex disorders, already because the hunt is for a single genetic factor. To date, several monogenic migraine syndromes are known (Fig. 3), some in which migraine is the main characteristic while in others it is one among other symptoms. Some of these other symptoms are more easily diagnosed or reported by the families such as stroke or dementia.

Several monogenic disorders are associated with an increased migraine risk. Among these disorders are a disease of the circadian clock, several cerebral small vessel diseases, mitochondrial disorders, and neuronal and glial disorders (Fig. 3). The cause of the higher prevalence of migraine in these disorders is not always understood.

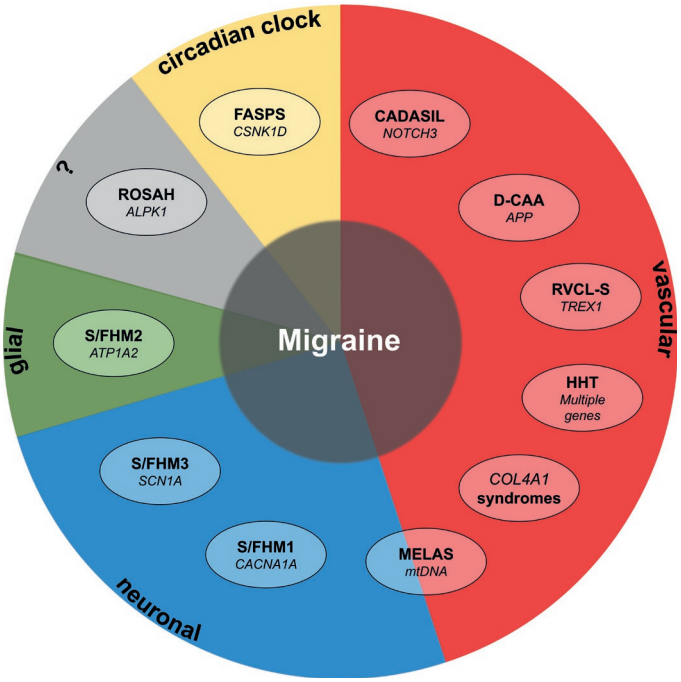

Figure 3. Pathophysiological pathways implicated in migraine and associated monogenic migraine syndromes.

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL); Dutch-type hereditary cerebral amyloid angiopathy (D-CAA); Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S); Hereditary hemorrhagic telangiectasia (HHT); *COL4A1*-related disorders; Mitochondrial myopathy with Encephalopathy, Lactic Acidosis, and Stroke (MELAS); Sporadic and Familial Hemiplegic Migraine type 1, 2 and 3 (S/FHM 1, 2, 3); Retinal dystrophy, Optic nerve edema, Splenomegaly, Anhidrosis and migraine Headache (ROSAH); and Familial Advanced Sleep-Phase Syndrome (FASPS).

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by mutations involving codons of cysteine residues in the *NOTCH3* gene. NOTCH3 plays an important role in vascular smooth muscle cell functioning.[64,65] Patients with CADASIL can experience recurrent ischemic stroke, apathy, mood disturbances and progressive cognitive decline leading to dementia as well as migraine. Disease onset is usually during mid-adulthood. Migraine with aura is the first clinical symptom in almost half of cases.[66] Atypical and typical migraine with aura attacks are reported by more than 50% of patients, and may also include motor aura symptoms. A distinction between a transient ischemic attack (TIA) and migraine aura might be difficult.[66]

Dutch-type Cerebral Amyloid Angiopathy

Dutch-type cerebral amyloid angiopathy (D-CAA, previously called Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type (HCHWA-D)) is caused by a single missense mutation (E693Q) in the Aβ region of the *APP* (amyloid precursor protein) gene. Patients with D-CAA may suffer from recurrent intracerebral hemorrhages at young age and frequently develop vascular dementia.[67] There is an increased prevalence of migraine with aura among mutations carriers. Strikingly, similar to CADASIL, migraine with aura may be the presenting clinical symptom in up to 80% of D-CAA patients.[68] Importantly, aura symptoms lasting ≥60 minutes signaled acute hemorrhage in 55% of cases.[68]

Hemiplegic migraine

Hemiplegic migraine is a subtype of migraine with aura (Table 2). Hemiplegic migraine can be subdivided into familial hemiplegic migraine (FHM) and sporadic hemiplegic migraine (SHM), depending on the presence or absence of relatives with hemiplegic migraine.[4]

Table 2. Criteria for hemiplegic migraine and its subtypes in accordance with the International Classification of headache disorders, third edition.[4]

ICHD-3 criteria for hemiplegic migraine
1.2.3 Hemiplegic migraine A. Attacks fulfilling criteria for migraine with aura B. Aura consisting of both of the following: 1. fully reversible motor weakness 2. fully reversible visual, sensory and/or speech/language symptoms.
1.2.3.1 Familial hemiplegic migraine (FHM) A. Attacks fulfilling criteria for hemiplegic migraine B. At least one first- or second-degree relative has had attacks fulfilling criteria for hemiplegic migraine
1.2.3.2 Sporadic hemiplegic migraine (SHM) A. Attacks fulfilling criteria for hemiplegic migraine B. No first- or second-degree relative fulfils criteria for hemiplegic migraine

It is characterized by a transient hemiparesis during the aura phase, which may last up to hours or even days. Notably, in hemiplegic migraine the visual and sensory aura symptoms are identical to those seen in common migraine with aura, although the duration is often longer.[69] Nevertheless, several symptoms have been reported to occur more frequently in hemiplegic migraine compared to migraine with aura. Patients with hemiplegic migraine are more likely to experience two or more aura symptoms, to suffer from prolonged aura symptoms (up to days, weeks or even months) and symptomatology indicating to brainstem involvement with dysarthria, vertigo, tinnitus, hypoacusis, diplopia, ataxia and decreased level of consciousness.[70,71] The diagnostic value of these symptoms has not been investigated. Importantly, the majority of patients also experience attacks of migraine with aura without paresis or migraine without aura in addition to their hemiplegic attacks.[72,73]

The chance of finding a causal mutation in hemiplegic migraine patients is higher in those with a lower age of onset, when there are more affected family members, and when attacks exhibit the following characteristics: (1) extensive motor weakness, (2) trigger with mild head trauma, (3) include confusion, (4) manifest symptoms of brainstem pathology, or (5) are associated with brain edema or cerebral spinal fluid (CSF) pleocytosis. Notably, mental retardation and progressive ataxia are only found in patients with a causal mutation in one of the three known hemiplegic migraine genes.[74] As such, phenotypic features in hemiplegic migraine patients may guide physicians in selecting cases for mutation screening and in providing adequate genetic counseling.[74] Physicians should also realize that disease severity may differ based on specific mutations and which of the known hemiplegic migraine genes is involved. As example, the p.Ser218Leu *CACNA1A* mutation is associated with severe attacks and cerebral oedema, seizures and coma, even up to attacks being fatal.[75] Mental retardation and brain atrophy have also been described as a consequence of hemiplegic migraine attacks.[76]

Hemiplegic migraine: pathophysiology

A considerable subset of hemiplegic migraine patients has a monogenic autosomal dominant form that can be caused by a single highly penetrant mutation in genes implicated in FHM1-3. Specific missense mutations in the *CACNA1A* gene, located on chromosome 19p13, cause FHM1. This gene is responsible for encoding the alpha-1A subunit of the $Ca_V2.1$ (P/Q type) calcium channel. FHM2 is caused by distinct missense mutations in the *ATP1A2* gene on chromosome 1q23. *ATP1A2* encodes a catalytic subunit of the alpha-2 subunit of the sodium/potassium ATPase. Finally, FHM3 is caused by specific missense mutations in the *SCN1A* gene on chromosome 2q24. *SCN1A* encodes a transmembrane alpha-1 subunit of the brain $Na_V1.1$ sodium channel.

For all three hemiplegic migraine types of knock-in mouse models have been generated by expressing pathogenic human gene mutations in the respective endogenous mouse gene. FHM1 mutant mice with either the R192Q or the S218L missense mutation revealed increased neuronal calcium influx and enhanced (cortical) excitatory, but not inhibitory, neurotransmitter release.[77-80] FHM2 mutant mice with either the W887R or the G301R mutation demonstrated abnormal glutamate uptake through glial cells.[81-83] Moreover, for the W887R mutant a reduced rate of glutamate and K^+ clearance, and a reduced density of GLT-1a glutamate transporters in astrocytic processes surrounding glutamatergic synapses was shown which facilitated the ignition of experimentally induced CSD.[81,82] In line with these findings, both the FHM1 and FHM2 mutant mice also demonstrate an increased susceptibility for experimentally induced CSD.[14,79,81,84] Recently, the first FHM3 mouse model (expressing the L263V mutation) was generated that demonstrated, for the first time, *spontaneous* CSD events.[85] Interestingly, the waves of CSD consistently propagated from visual to motor cortex, corresponding to the clinical presentation in humans.[69]

Taken together these findings predict increased neurotransmitter and potassium ion levels *in vivo* at the synaptic cleft, which facilitates CSD and explains the aura features in patients with these mutations.[86-91] Moreover, FHM1 mice also demonstrate an enhanced susceptibility to ischemic depolarizations which could be the underlining mechanism predisposing migraineurs to infarction during mild ischemic events, thereby increasing the stroke risk.[92,93]

Several studies have been conducted in humans to further understand the pathological mechanisms causing hemiplegic migraine. As *CACNA1A* is also expressed at the neuromuscular junction, neuromuscular function was evaluated in FHM patients with *CACNA1A* mutations using single-fibre electromyography (EMG). Mean jitter did not differ between patients and control subjects or among patients and no blocking was found.[94] These results suggest that neuromuscular function is normal in FHM. With an exploratory ^{1}H-NMR metabolomics analysis lower levels of 2-hydroxybutyrate were found in patients with hemiplegic migraine.[95] This may indicate a dysregulation of the brain's energy metabolism. Another indication of involvement of energy metabolism came from a study using ^{31}P magnetic resonance spectroscopy (^{31}P-MRS). A higher concentration of adenosine diphosphate in the brains of the patients with familial hemiplegic migraine was found.[96] Another MRS study that used a high-field 7 tesla brain proton MRS (^{1}H-MRS) demonstrated a decreased cerebellar N-acetylaspartate/total creatine ratio in the absence of macroscopic atrophy and regardless of signs of cerebellar dysfunction in hemiplegic migraine patients.[97] This suggests that cerebellar neuronal loss or dysfunction occurs in hemiplegic migraine. The likelihood of inducing a migraine-like

attacks after nitroglycerin and CGRP administration is generally increased in patients with migraine without aura compared with patients with migraine with aura, and in migraineurs with high attack frequency compared with migraineurs with low attack frequency.[98,99] Patients with FHM typically have a much lower attack frequency than patients with migraine without aura, predicting a lower response rate to nitroglycerin or CGRP provocation. Indeed, the incidence of migraine-like headaches after provocation with nitroglycerin or CGRP was lower in patients with FHM than in patients with common types of migraine.[100-104] These results might point to different starting points for attack initiation for hemiplegic migraine compared to common migraine.

Not all hemiplegic migraine patients will be found to have a causal mutation in one of the known FHM1-3 genes.[69,74,105] Several genes have been suggested as the possible fourth hemiplegic migraine gene.[106] Nonetheless, whole exome sequencing (WES) has failed to identify undisputed pathogenic mutations in additional genes that fit a Mendelian inheritance pattern.[74] A major fourth autosomal dominant gene for hemiplegic migraine may therefore be unlikely, although this cannot be fully excluded. Physicians should realize that this means that not all offspring of hemiplegic migraine cases have an *a priori* 50% chance to inherit hemiplegic migraine. Genetic counseling may be helpful to clarify estimated risks.

PRRT2 has been suggested as the fourth hemiplegic migraine gene.[107] While their appears to be an association between hemiplegic migraine and *PRRT2*, the vast majority of *PRRT2* carriers with the presumed causative mutation do not have (hemiplegic) migraine attacks. Moreover, large hemiplegic migraine families in which a *PRRT2* mutation has been found often have many non-penetrant cases or it is later found out that there is an additional mutation in one of the well-established FHM1-3 genes.[74,108] Thus, *PRRT2* more likely seems to act as a genetic modifier of (hemiplegic) migraine risk. Therefore, in clinical practice neurologists and geneticists who find a *PRRT2* mutation in a hemiplegic migraine patient should be careful to claim genetic confirmation of a clinical diagnosis and should be aware the (hemiplegic) migraine will not be inherited in an autosomal dominant manner. Several other genes have been suggested as possible hemiplegic migraine genes (i.e., *SLC1A3* and *SLC4A4* [109-111]) but lack independent confirmation.

Most hemiplegic migraine patients without a mutation in *CACNA1A*, *ATP1A2*, or *SCN1A* display a mild phenotype without additional symptomatology, and may seem to be more like those with common migraine. It is possible that other genetic mechanisms play a key role in these cases. Possibly, a non-autosomal dominant mechanism could be involved in which rare gene variants with intermediate effect sizes lead to the disorder.

This is, however, extremely difficult to study as it is unclear to what extend reduced penetrance is implicated and how many variants might play a role. This can even be up to a point where complex polygenic interactions involving multiple gene variants with small effect sizes may occur, as is the case for the common migraine types. Some evidence for this theory exists. In a large study that used polygenic risk scoring, based on genetic information of common variants from the 2016 migraine GWAS,[24] it was shown that both familial migraine with aura and hemiplegic migraine cases (without a mutation in the known genes) had higher polygenic risk scores compared to cases of familial migraine without aura.[105] This observation implies the likelihood of common variants, and the possibility these variants are playing a significant role in hemiplegic migraine when no mutation is found in the FHM1-3 genes.

Hemiplegic migraine: management

Due to the rarity of hemiplegic migraine, no large randomized controlled trials can be performed in this patient group. There are no targeted treatments for patients with specific mutations, although precision medicine remains an elusive ideal. Treatment largely follows guidelines for the common form of migraine with aura. Preference for certain drugs is mostly based on data from migraine with aura and expert opinion.

In hemiplegic migraine attacks the aura is often more debilitating than the headache. Acute migraine medication may alleviate headache, nausea, photophobia but no acute medication for treating the aura phase is available. First choice in acute treatment usually is acetaminophen or an NSAID. If not effective, triptans can be prescribed. There is no experience with the newer drug treatments, ditans and gepants. But theoretically these will only work on the headache phase similar to the current medication options.

Migraine aura was previously thought to be caused by ischemia, but regional cerebral blood flow studies dispelled that idea.[112] Sumatriptan-induced vasoconstriction occurs only in the dilated vessels, without affecting normal ones.[113] Even so, because hemiplegic migraine patients have severe aura, triptans were officially contraindicated. However, extensive clinical experience has shown that triptans can safely be used in both hemiplegic migraine and migraine with aura and other monogenic small vessel diseases, such as CADASIL. Retrospective studies in hemiplegic migraine patients showed that side effects were rare and minor.[114-116] There are only few reports on preventive treatments in hemiplegic migraine. Abortive treatment with verapamil and acetazolamide was described as incidentally effective.[117] Furthermore, ketamine has been reported to show some improvement as attack treatment in a small sample size. This is especially interesting as NMDA-receptor antagonists blocked CSD in

animal studies.[118] However, as ketamine may cause dependency and tolerance with prolonged use and should therefore not be recommended.

Preventive treatment is mostly prescribed for high-frequency migraine. In hemiplegic migraine it may also be considered for patients with low frequent but severe attacks. Acetazolamide, flunarizine, lamotrigine, sodium valproate and verapamil can be tried in no strictly preferred order.[117] Other migraine prophylactics, such as candesartan, pizotifen, propranolol and topiramate might also be considered, although there is less evidence for efficacy.[117] When deciding on treatment to start it should be taking into account that hemiplegic migraine patients have an increased risk of developing depressive symptoms, so this should be carefully monitored.[119] Hemiplegic migraine attacks can be triggered by (minor) head trauma.[74] Therefore, patients that have experienced trauma-triggered attacks should be advised not to practice contact sports. No evidence-based advice is available on other lifestyle or dietary factors.

Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations

Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic Manifestations (RVCL-S) is an autosomal dominant inherited angiopathy caused by mutations in the *TREX1* gene (Table 3).[120,121] Worldwide less than 30 families with RVCL-S are known, so far.[120,122-132] However, this is expected to be an underestimation given that in the Netherlands alone, three unrelated families have been identified.[120] Moreover, RVCL-S is often mistaken for other disorders. With more awareness and new genetic screening techniques, such as whole exome sequencing, new cases and more families will be identified.

Table 3. RVCL-S diagnostic criterium and main features.[133]

Diagnosing and suspecting RVCL-S
Diagnostic criterium A. C-terminal frameshift mutation in TREX1
Main features A. Vascular retinopathy B. Features of focal and/or global brain dysfunction associated on MRI with C. punctate T2 hyperintense white matter lesions with nodular enhancement D. T2 hyperintense white matter mass lesions with rim-enhancement, mass effect, and surrounding edema E. Family history of autosomal dominant inheritance with middle-age onset of disease manifestation
Supportive features A. On CT focal white matter calcifications and/or on MRI nonenhancing punctate T2 hyperintense white matter lesions at an age that non-specific age-related white matter hyperintensities are infrequent B. Microvascular liver disease C. Microvascular kidney disease D. Anemia consistent with blood loss and/or chronic disease E. Microscopic gastro-intestinal bleeding F. Subclinical hypothyroidism

RVCL-S patients often suffer from retinal vasculopathy, focal and global neurological symptoms and systemic manifestations (e.g., impaired renal and liver function).[134-136] In RVCL-S, migraine occurs more frequently than in the general population.[135,136] In affected individuals migraine onset appears later in life (>40 years), which might suggest that migraine is a secondary phenomenon caused by the progressive cerebral vasculopathy.[135,137] As such, the cause of the migraine attacks might be different than in the case of CADASIL or Dutch-type cerebral amyloid angiopathy (D-CAA), where migraine is often an early clinical sign.[66,68] The main causes of morbidity and mortality in RVCL-S are vascular retinopathy, focal neurological complaints including ischemic events and cognitive decline, and kidney failure.[120]

RVCL-S: pathophysiology

RVCL-S is caused by carboxyl terminal (C-terminal) truncating mutations in the *TREX1* gene (encoding 3-prime repair exonuclease 1).[120,121] The underlying lesion is a non-atherosclerotic, amyloid-negative angiopathy involving small arteries and capillaries, primarily in the retina and brain but also in other organs.[120,135]

In mammals TREX1 is a major 3'-5'-exonuclease with high affinity for single-strand DNA and it is responsible for the removal of nucleoside monophosphates from the 3'-ends of DNA.[138,139] The TREX1 protein is highly conserved among species and its N-terminus contains three exonuclease domains while its C-terminal domain is required for its localization to the endoplasmic reticulum.[121,122,138,140,141] The mutations leading to RVCL-S result in a truncated TREX1 protein. The exonuclease function of TREX1 is not affected. However, the subcellular localization is altered.[121,122,141] Additionally, in lymphoblasts of RVCL-S patients an increase in free glycan release was demonstrated, most likely due to altered oligosaccharyltransferase activity.[141] Furthermore, in a transgenic mouse model of RVCL-S (with the V235*fs* mutation) a distinct autoantibody profile was found compared to that of wildtype mice.[142]

Morphological pathological studies have demonstrated thicker multilaminated basement membranes in RVCL-S patients.[120] Also, fibrous thickening of small vessel walls has been demonstrated.[120] Moreover, TREX1 was found to be expressed in a subset of microglia in the normal human brain, often in close proximity to the microvasculature. Interestingly, in ischemic lesions the amount of TREX1 positive microglia was increased.[130,143] This indicates a possible role for TREX1 and microglia in vessel homeostasis and response to ischemic injury. In the undamaged white matter of RVCL-S patients there is also an increased expression of TREX1, suggestive for a widespread ongoing injury that cannot be detected histologically.[130,143] Moreover, TREX1 was found to be expressed in endothelial cells in the brain of a RVCL-S patient.[130]

Experimental studies in in-house generated RVCL-S transgenic knock-in mice (also with the V235*fs* mutation) have shown increased mortality, signs of abnormal vascular function, and increased sensitivity to experimental stroke and can be instrumental to investigate the pathology seen in RVCL-S patients.[144]

RVCL-S: management

There is no specific disease-modifying treatment for RVCL-S. A pilot study for treating RVCL-S with aclarubicin was started but not finished due to serious side effects of the treatment. Limited information is available regarding the management of the major manifestations of the disease. Acute transient ischemic attack and acute stroke in patients with RVCL-S are managed following the general principles of stroke medicine. However, there is no proof that intravenous thrombolytic therapy (IVT) is effective and as RVCL-S is an angiopathy it may lead to increased bleeding risk. Therefore, IVT is not recommended similar to CADASIL. For secondary stroke prevention, we suggest that in patients with RVCL-S and a symptomatic ischemic event which has been confirmed by MRI imaging, available risk reduction strategies should be taken into consideration, including antiplatelet therapy, and statin treatment although specific evidence of effectiveness for RVCL-S is lacking. In addition, smoking cessation may be particularly important. Other suggested lifestyle modifications include limited alcohol consumption, no drugs, weight control, regular physical activity, and a Mediterranean diet that is rich in fruits, vegetables, and low-fat dairy products. We suggest measures to control blood pressure, cholesterol, glucose, and weight for all patients with RVCL-S on a regular basis.

If there are significant signs/symptoms of a specific organ patients should be referred to a specialist for that specific organ. However, experts with knowledge about RVCL-S advisable to avoid unnecessary diagnostics procedures (such as biopsies) as to prevent under- or overtreatment. Furthermore, when a patient is first diagnosed, a consultation with a clinical geneticist with knowledge about RVCL-S is recommended and other family members should be offered the possibility of genetic counseling.

It is advisable to perform a yearly check-up with neurological, ophthalmological, and general evaluation from diagnosis onwards to detect possibly treatable complications and symptoms. If symptoms are occurring and severe, more frequent monitoring may be necessary.

Most symptoms of RVCL-S can be treated with usual care for those specific organs. Retinopathy and macular edema can be treated with retinal laser therapy and intravitreal anti-VEGF. Glaucoma, hypertension, migraine, seizures, hypothyroidism,

anemia, Raynaud phenomenon and psychiatric complaints can be treated according to standard guidelines. For the treatment of anemia in severe cases intravenous blood transfusions may be necessary. In case of renal disease, control of hypertension is especially important. If cerebral vascular edema is present (especially common with pseudotumors) corticosteroid therapy should be considered (intravenous methylprednisolone, followed by oral corticosteroid treatment).

Asymptomatic family members should be offered genetic counseling prior to procedures that could detect signs of RVCL-S. Predictive testing and specific mutation testing is possible, once the *TREX1* pathogenic variant has been identified in an affected family member. Potential consequences of such a test should be discussed (socioeconomic consequences, long term follow-up). However, RVCL-S differs from many hereditary neurological diseases as there are health advantages to early diagnosis. Genetic screenings can identify individuals who will benefit from prompt initiation of treatment to preventive or delay complications of organ damage due to affected small vessels. Evaluation of signs of retinopathy is especially important as treatment can be necessary before onset of ocular complaints and can prevent early blindness at the age of 50 years.

Equality in research

Embracing diversity in the workplace further enhances team well-being and provides a distinct competitive advantage.[145-147] Likewise, a diverse and equal representation of researchers fosters innovation and creativity as different perspectives and experiences contribute to a broader understanding of complex issues.[147] As an example, publications authored by groups with ethnic and gender diverse authors attract significantly more citations than those written by researchers of a single ethnic group or gender.[148] A closer examination of these publications still reveals significant disparities. Despite the noticeable increase in the contribution of women as first authors of original research in high-impact medical journals over the past 20 years, progress has plateaued in recent years.[149]

Outline of this thesis

The research conducted for this thesis is divided in three parts. **Part I** of this thesis focuses on the genetics of migraine. Here migraine is investigated by: (i) reviewing our current understanding of the genetics of the migraine aura, (ii) an untargeted

epigenome-wide approach aimed at understanding migraine chronification, (iii) the prevalent neurological and psychiatric comorbidities and how they are genetically linked to migraine and (iv) how patients with hemiplegic migraine and monogenic migraine syndromes respond to migraine treatment. **Part II** of this thesis focusses on vascular monogenic migraine syndromes, there disease mechanism and possible biomarkers. Finally, **Part III** focusses on the challenges physicians and researchers may face in the headache field and are important to address to ensure that the future of headache research and clinical practices is ensured. This part is aiming to make recommendations for the (inter)national headache field.

Part I: Migraine – insights into genetics, epigenetics, comorbidities, and monogenic factors

In **Chapter 2** an overview of the genetics of migraine aura is given. **Chapter 3** investigates whether DNA methylation changes occur when patients with chronic migraine and medication-overuse respond to treatment. To this end, a longitudinal epigenome-wide association study was conducted where patients with chronic migraine were treated with withdrawal. Baseline methylation status was compared with methylation status after three months of treatment. **Chapter 4** describes neurological and psychiatric comorbidities associated with migraine and how genetics plays a role in this. In **Chapter 5** we investigated how patients with hemiplegic migraine and monogenic migraine disorders (CADASIL and RVCL-S) respond to migraine treatments.

Part II: Monogenic small vessel diseases – understanding vascular migraine models

In **Chapter 6** we discuss the importance of considering safety when treating vascular monogenic migraine, especially given the new migraine treatments targeting the CGRP system. **Chapter 7** explores the hypothesis the RVCL-S and CADASIL are caused by different disease mechanisms involving the endothelium and vascular smooth muscle cells, respectively. The retina is considered an extension of the central nervous system (CNS) and shares structural and functional similarities with the brain. Studying the retina provides a unique opportunity to gain insights into neurological conditions. Additionally, useful biomarkers are currently not available for these disorders. In **Chapter 8** and **Chapter 9** we therefore investigated whether RVCL-S and D-CAA lead to changes in retinal thickness using optical coherence tomography (OCT). In **Chapter 10** we evaluated the vasculature of RVCL-S in more detail using OCT angiography (OCT-A) and in **Chapter 11** we investigated to role of the oxygen metabolism by way of oximetry. These techniques, each non-invasive and user friendly might provide useful biomarkers in the future. In **Chapter 12** we characterized the neuropsychiatric

phenotype of RVCL-S patients and evaluated whether MRI characteristics are related to cognitive impairment, psychiatric morbidity and apathy.

Part III: Equality in headache research – tackling career barriers and harassment

To promote clinical and research opportunities in the headache field based solely on abilities and to remove possible career barriers providing an unfair disadvantage, we performed a global assessment of career barriers (**Chapter 13**) and harassment (**Chapter 14**).

A general discussion of the findings presented in this thesis as well as suggestions for future research are given in **Chapter 15**.

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