Monogenic models of migraine : from clinical phenotypes to pathophysiological mechanisms

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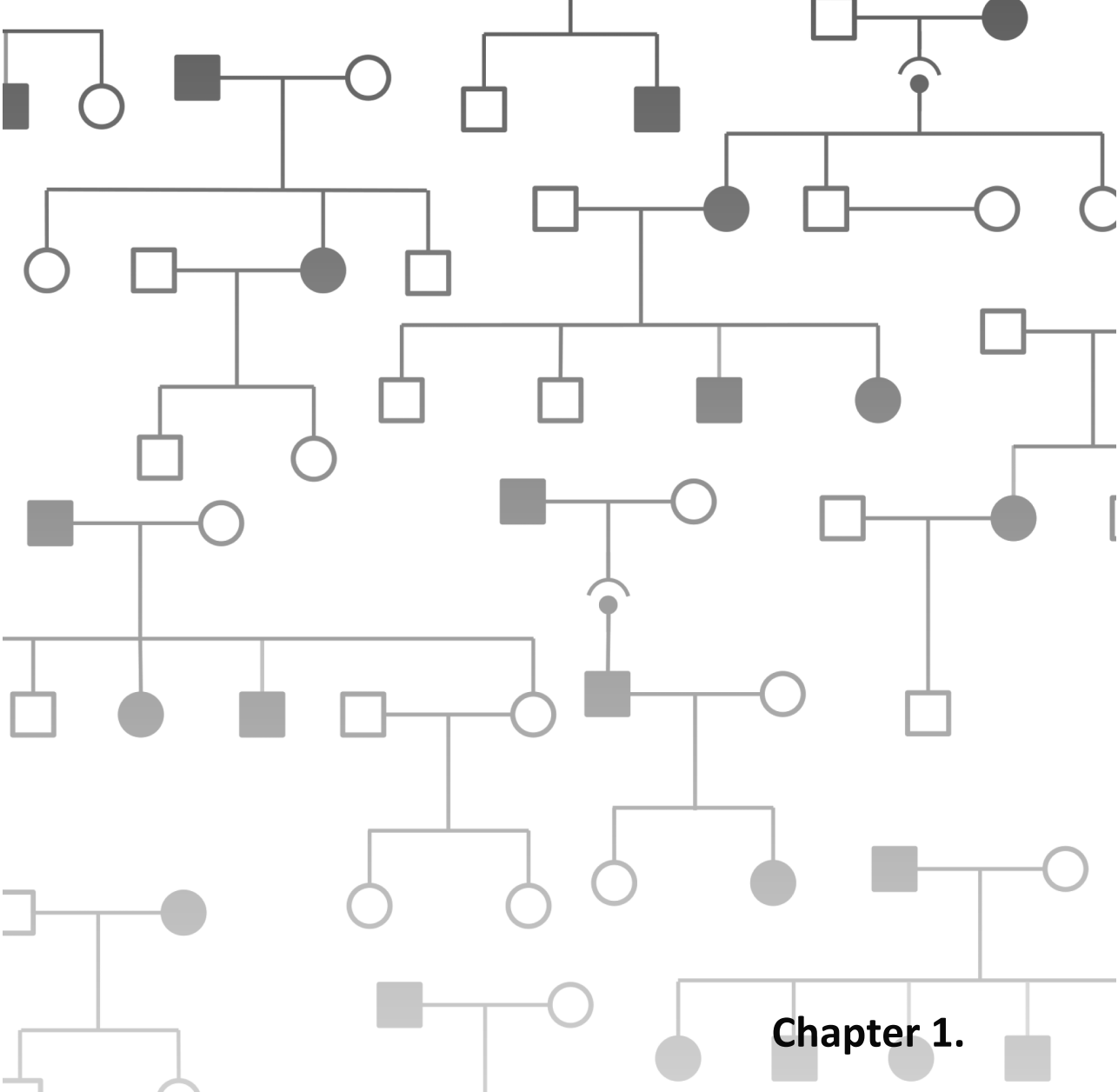

Chapter 1.

General introduction

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Hemiplegic Migraine and Other Monogenic Migraine Subtypes and Syndromes.

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General introduction

Clinical characteristics of migraine

Migraine is a disabling episodic brain disorder characterised by headache attacks associated with photophobia and phonophobia and/or nausea or vomiting.[1] A typical migraine headache lasts 4–72 hours, is one-sided, of pulsating quality and of moderate to severe intensity. Intensity of the headache often increases with physical exercise, restricting patients to bed. The majority of migraine patients experience premonitory symptoms such as fatigue, loss of concentration or mood disturbances in the 24 hours preceding the attack.[2] In approximately one-third of patients headaches are preceded by transient focal neurological symptoms, the so-called auras, such as scotomas and scintillations, and, less frequently, paraesthesiae or dysphasia. Aura symptoms develop gradually, i.e. the symptoms become more extensive over several minutes, or different types of auras occur sequentially.[3] Each individual aura symptom generally last 5–60 minutes.

Due to the lack of reliable biomarkers that could serve as the gold standard for diagnosing migraine, the diagnosis is based on clinical criteria from the International Classification of Headache Disorders (ICHD) of the International Headache Society (IHS) (Table 1).[1] As many of the criteria for migraine in the ICHD are optional, different combinations of symptoms can result in a migraine diagnosis. The presence of aura symptoms defines the two main migraine types: migraine with aura and migraine without aura.

One of migraine's most striking features is its high prevalence, especially in women. Studies from throughout the world consistently report lifetime migraine prevalence to range from 19-34% in women and from 8–18% in men.[4–11] Taking all characteristics of migraine into consideration, it comes as no surprise that migraine ranked at number eight on the most recent list of global leading causes of years lived with disability.[12] The burden of migraine clearly needs lifting, but with the available acute anti-migraine drugs sustained pain free rates are generally below 30%[13] and with prophylactic drugs significant (>50%) reduction of attack frequency is on average achieved in less than 50% of patients.[14] A good understanding of migraine pathophysiology is needed to identify novel drug targets. Given the wide variability of clinical phenotypes, one could hypothesise that there are differences in pathophysiological mechanisms among different migraine subtypes.

Pathophysiology of migraine

Migraine is considered a disease with involvement of both neuronal and vascular mechanisms. A major leap forward in the understanding of the pathophysiology of migraine with aura was the

Table 1: Criteria for migraine with and without aura according to the International Classification of Headache disorders, third edition (ICHD-3), beta version.[1]

ICHD-3 criteria
1.1 Migraine without aura **A.** At least five attacks fulfilling criteria B–D **B.** Headache attacks lasting 4–72 hours (untreated or unsuccessfully treated) **C.** Headache has at least two of the following four characteristics: **1.** unilateral location **2.** pulsating quality **3.** moderate or severe pain intensity **4.** aggravation by or causing avoidance of routine physical activity (e.g. walking or climbing stairs) **D.** During headache at least one of the following: **1.** nausea and/or vomiting **2.** photophobia and phonophobia **E.** Not better accounted for by another ICHD-3 diagnosis.
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 and/or language **4.** motor **5.** brainstem **6.** retinal **C.** At least two of the following four characteristics: **1.** at least one aura symptom spreads gradually over ≥5 minutes, and/or two or more symptoms occur in succession: **2.** each individual aura symptom lasts 5–60 minutes1 **3.** at least one aura symptom is unilateral **4.** the aura is accompanied, or followed within 60 minutes, by headache **D.** Not better accounted for by another ICHD-3 diagnosis, and transient ischaemic attack has been excluded.

discovery of the phenomenon Cortical Spreading Depolarisation (CSD), a wave of neuronal and glial depolarisation that slowly propagates across the cerebral cortex at 3–5 mm per minute and is followed by a prolonged phase of nerve cell depression.[15,16] The initial excitatory phase (the wave front) is thought to be linked to positive aura symptoms (e.g. scintillations, feelings of pins and needles), the inhibitory phase (the depression) can be associated with negative aura symptoms (e.g. scotomas, numbness). A strong indication that CSD can occur in humans was provided by a functional MRI study measuring blood flow changes in the visual cortex.[17] Another imaging study in migraine suggested that a short phase of increased blood flow is followed by a period of decreased blood flow, which could be associated with the phases of depolarisation and depression of CSD[18], but this was not shown in other studies.[19–21] Nonetheless, it is widely assumed that vascular mechanisms, linked to CSD, are involved in migraine with aura.[22] Data from experimental animals suggest that CSD is involved in the activation and sensitization of the trigeminovascular system, which consists of connections of meningeal vessels and brainstem centres[23], that involves release of (vasoactive)

neuropeptides, such as calcitonin gene-related peptide (CGRP) and eventually causes pain. As CSD is closely linked to the migraine aura, it remains unclear whether CSD could also be involved in migraine without aura, and if so, how the aura would remain 'silent' in these patients.[22,23] Alternatively, in migraine without aura other mechanisms are involved in activating the trigeminovascular system and causing the headaches.

Indications that vascular mechanisms may play a role in migraine not only arise from their involvement in activating the trigeminovascular system, but also from investigating comorbid diseases of migraine involving vascular dysfunction.[24] Most notable is the increased risk for ischemic stroke, which is most apparent in patients with migraine with aura.[25] More controversial is the association of migraine with haemorrhagic stroke, but some evidence for an increased risk in migraine patients was found.[26] Apart from these comorbidities, evidence for cerebral vascular pathology was found in migraine patients without a clear history of cerebrovascular events, which revealed an increased prevalence of white matter lesions and infarcts in the posterior circulation.[27] These lesions were clinically silent, and although progression was observed, this appeared to occur independently of migraine attack frequency.[28] Altogether it remains largely unclear how the increased prevalence of cerebrovascular pathology in migraine occurs. There may be a direct relation between cerebral hypoperfusion during CSD and vascular damage afterwards.[27] On the other hand, an increased tendency towards cervical artery dissections was found in migraine patients who suffered from an ischemic stroke, suggesting more widespread, extracerebral disruption of vascular integrity.[29,30] Besides a direct causal relationship, common underlying genetic or environmental risk factors and thus common pathophysiological mechanisms form a plausible explanation for the observed comorbidity. One possible common mechanism is endothelial dysfunction, which has been suggested to occur in migraine patients.[31] For example, markers of endothelial activation were found to be increased in premenopausal women with migraine,[32] and biomarkers of hypercoagulability and inflammation have been associated with migraine in a population-based study.[33] Circulating endothelial progenitor cells (EPC) are suggested to provide valuable information about endothelial regeneration capacity.[34] Although the cultured EPC type that probably best reflects 'real' EPCs has not been investigated in migraine, circulating numbers and function of an 'EPC-like cell type' have been proposed to be reduced in migraine, suggesting that migraine patients may exhibit dysfunctional endothelial regeneration.[35,36] In addition, levels of endothelial microparticles were shown to be elevated in migraine with aura patients, suggesting increased endothelial activation.[37] Evidence of systemic endothelial dysfunction could not always be demonstrated, which implies that endothelial dysfunction in migraine may be limited to the cerebral circulation.[38,39] As is illustrated by the mentioned studies on endothelial function, many different methods can be applied when trying

to identify novel pathophysiological mechanisms.[32,33,35–39] Aside from biochemical research, functional biometrics or imaging techniques, an important contribution to pathophysiological research can be provided by genetic research, which has been an especially important field in migraine research.

Genetics of migraine

Migraine with aura and migraine without aura both have a strong genetic component.[40-43] Family studies consistently revealed an increased familial risk of migraine, which seemed more pronounced for migraine with aura.[41] From these studies it became apparent that inheritance of migraine generally does not follow autosomal dominant patterns, but rather involves complex genetic mechanisms.[44] In complex diseases, (multiple) genetic factors confer susceptibility to the disease, but are by themselves of small effect size and not sufficient to cause the disease. Environmental factors also play a role in the development of migraine. Nonetheless, identifying genetic factors involved can provide important clues about disease pathophysiology. A challenge for all genetic studies of the common forms of migraine (i.e. migraine with aura and migraine without aura) is the considerable clinical heterogeneity, which can be tackled by either studying large numbers of patients or by restricting the phenotypic spectrum that is studied. In migraine genetics, both approaches have been applied successfully.[14,45]

Complex genetics of common migraine subtypes

Because migraine is a very common disease, it is expected that the genetic factors involved in migraine are also quite common. In the past, researchers attempted to identify genes involved in the common forms of migraine using case-control studies.[45] Genes were selected based on knowledge of pathways (mostly serotonin and dopamine pathways) that were presumed to be important in migraine pathophysiology. These candidate gene association studies in migraine for the most part were quite small and investigated only one, or at best a few, genetic variants per gene. The statistical power of these studies was, therefore, limited, and even if associations were found, these were rarely replicated in independent cohorts.

In the last five years, another approach, namely genome-wide association (GWA) studies, has turned out to be successful in identifying common genetic variants for complex diseases. In GWA studies, a large number of single nucleotide polymorphisms (SNPs) (hundreds of thousands of variants) is genotyped in a large number of patients and controls (thousands per group), and the prevalence of each allele of a SNP is compared between both groups. The SNPs of which the allele frequency

difference reaches the genome-wide significance threshold (p-value $<5x10^{-8}$) are considered to be associated with the disease.[46] A complication in the interpretation of virtually all findings from GWA studies is that the associated SNPs are not located in coding regions but rather in intergenic and intronic regions.[47] Linking these SNPs to genes is therefore a challenging task. For example, a SNP may actually be located in a regulatory region of a gene that is much further away than the gene in closest proximity. More research on how to interpret the results of GWA studies is essential, as the ability to correctly determine which genes are involved in a disease provides a means to also identify the correct pathophysiological pathways these genes are involved in.[48] GWA studies have led to the identification of multiple genetic risk factors for migraine.[49–52] However, due to the low effect size of the disease-associated SNPs, they only explain a small percentage (at best 20%) of the total heritability of migraine.[47,53] A large portion of the heritability and its associated pathophysiological pathways thus remain to be discovered, and it is questionable whether simply increasing the size of GWA studies will solve this issue.

Monogenic models of migraine

Another strategy is to study monogenic forms of migraine, such as hemiplegic migraine (HM), a rare monogenic subtype of migraine with aura.[1] Furthermore, there are monogenic syndromes that are not primarily migrainous disorders but show association with migraine in different ways (Figure 1). First, several monogenic syndromes are caused by mutations in genes that have been associated with HM, but these mutations are either of a different type than found in HM or have different functional consequences. Examples are *CACNA1A*-associated disorders (Spinocerebellar Ataxia type 6 (SCA6)[54] and Episodic Ataxia type 2 (EA2)[55]) and *SCN1A*-associated epilepsy syndromes (severe myoclonic epilepsy of infancy (SMEI)[56] or generalised epilepsy with febrile seizures (GEFS+)[57]). Second, symptoms of some monogenic syndromes can mimic HM, such as the migraine-like headache and neurological deficits in Mitochondrial myopathy with Encephalopathy, Lactic Acidosis, and Stroke (MELAS) syndrome[58,59] or the paroxysmal hemiplegia in Alternating Hemiplegia of Childhood (AHC).[60] Studying the overlapping and diverging characteristics of these disorders and HM can reveal pathophysiological mechanisms that are also relevant to migraine. Finally, migraine can be part of the clinical spectrum of several monogenic (vascular) syndromes, in which a higher prevalence of migraine is observed than would be expected based on population risk. These syndromes include Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)[61], Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S)[62,63] and small-vessel diseases associated with mutations in *COL4A1.*[64] Other symptoms of these disorders are primarily vascular, and unravelling

pathophysiological mechanisms of these disorders may reveal vascular mechanisms that also play a role in migraine. As these syndromes are caused by a single gene mutation, there is a clear biomarker available to designate a subject as affected. In addition, it is more likely that similar pathogenetic mechanisms are involved in patients of such a family with a monogenic background than would be the case in patients with common migraine subtypes with variable complex genetic backgrounds. All in all, the more homogeneous clinical phenotype and knowledge of the gene that causes the syndrome may greatly facilitate the identification of pathophysiological mechanisms of the comorbid condition migraine. Thorough characterisation of the migraine phenotypes in the monogenic diseases is indispensable to determine which components of migraine are best modelled by each monogenic disease.

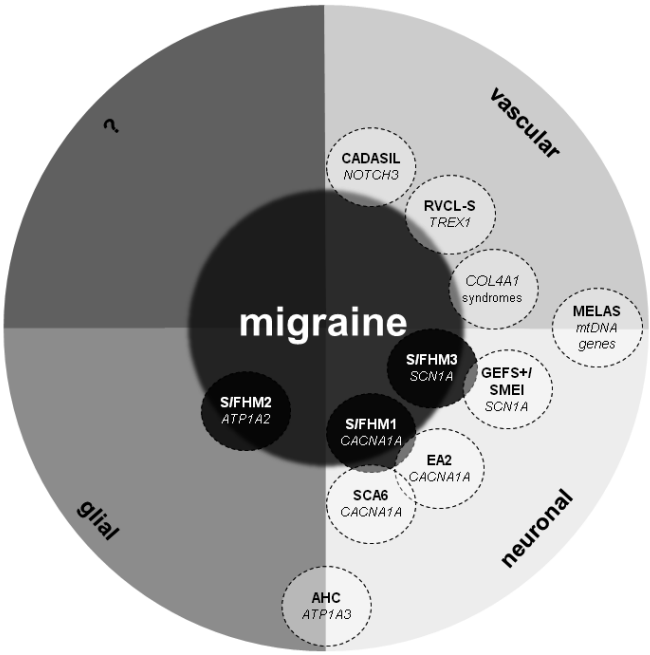

Figure 1: Monogenic syndromes associated with migraine. Depicted are relations of these monogenic syndromes with migraine and each other, causative genes (in italics) and the main associated pathophysiological pathways: Sporadic and Familial Hemiplegic Migraine type 1, 2 and 3 (**S/FHM1, 2, 3**), and allelic disorders Spinocerebellar Ataxia Type 6 (**SCA6**), Episodic Ataxia Type 2 (**EA2**) and generalised epilepsy with febrile seizures and severe myoclonic epilepsy of infancy (**GEFS+, SMEI**) affecting glial and neuronal ion channels; vascular syndromes Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (**CADASIL**), Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (**RVCL-S**), and monogenic syndromes associated with ***COL4A1*** mutations, and migraine-mimicking disorders Mitochondrial myopathy with Encephalopathy, Lactic Acidosis, and Stroke (**MELAS**, associated with mutations in mitochondrial DNA (mtDNA)) and Alternating Hemiplegia of Childhood (**AHC**).

Hemiplegic migraine: a neuronal and glial monogenic migraine model

Hemiplegic migraine (HM) is the only monogenic disorder with migraine as its key symptom. HM is considered as a subtype of migraine with aura in the ICHD (Table 2).[1] Familial and sporadic types of HM are recognised, with approximately 200 families and 200 sporadic cases described to date.[65] In Familial Hemiplegic Migraine (FHM) there is at least one first- or second-degree family member who also suffers from HM. In Sporadic Hemiplegic Migraine (SHM), the family history for HM is negative, but common migraine often occurs in family members.[1] HM shows an autosomal dominant inheritance, which means that offspring of an HM patient has a 50 percent chance to also inherit the genotype. SHM patients have often acquired a mutation *de novo* and thus a new FHM family may develop if the mutation carrier has offspring.[66]

Clinical characteristics of hemiplegic migraine

A clinical diagnosis of HM is based on the ICHD criteria (Table 2), which makes an interview or interpretation of a questionnaire by a physician the most important step of the diagnostic process.[1] Beside common aura symptoms and migraine headaches HM attacks include transient motor weakness varying from mild paresis to hemiplegia. HM patients have an increased risk for migraine with aura (55%) and without aura (25%).[67] Sometimes, patients find it hard to distinguish complaints of sensory disturbances from those of a mild paresis, because sensory auras may cause the sensation of being unable to grasp or lift objects and may be interpreted as mild motor auras.

Table 2: Criteria for Hemiplegic migraine according to the International Classification of Headache disorders, third edition (ICHD-3), beta version.[1]

ICHD-3 criteria
1.2.3 Hemiplegic[1] migraine
A. At least two attacks fulfilling criteria B and C
B. Aura consisting of both of the following:
1. fully reversible motor weakness
2. fully reversible visual, sensory and/or speech/language symptoms
C. At least two of the following four characteristics:
1. at least one aura symptom spreads gradually over ≥5 minutes, and/or two or more symptoms occur in succession
2. each individual non-motor aura symptom lasts 5–60 minutes, and motor symptoms last <72 hours[2]
3. at least one aura symptom is unilateral[3]
4. the aura is accompanied, or followed within 60 minutes, by headache
D. Not better accounted for by another ICHD-3 diagnosis, and transient ischaemic attack and stroke have been excluded.

Apart from the motor weakness, the symptoms and their order of appearance during attacks are very similar for HM and migraine with aura.[68] Other characteristics typical of HM are two or more aura

symptoms, aura symptoms of longer duration, and brainstem auras (dysarthria, vertigo, tinnitus, hypacusia, diplopia, ataxia and decreased level of consciousness).[1,68,69]

Differential diagnosis of hemiplegic migraine

Especially in the acute phase of a first HM attack, a transient ischemic attack (TIA) or stroke is often part of the differential diagnosis. A discerning factor is that migraine auras often start gradually, in contrast to the sudden onset of vascular events. When HM attacks always occur on the same side, underlying pathologies including arteriovenous malformations, arterial dissections and cerebral vasculitis must be excluded by MR imaging and/or MR angiography. During or shortly after an HM attack, diffuse (cortical) oedema of the symptomatic hemisphere is the most prevalent observation.[70–73] A few imaging abnormalities can support the diagnosis of HM in the interictal phase. In HM patients with progressive cerebellar ataxia and/or dysarthria, cerebellar atrophy has been described.[74,75] Other reported persistent imaging abnormalities include diffuse cortical and subcortical hyperintensities on T2-weighted MRI,[71,76,77] signs of ischemic necrosis,[78] or cortical cerebral atrophy[71,76,77,79] in previously affected hemispheres.

Epileptic features have been described during an HM attack and HM patients may experience separate migraine attacks and epileptic seizures. It may be difficult to distinguish migraine auras from epileptic phenomena, but electroencephalography (EEG) can be helpful.[80–82] EEGs during and after HM attacks have shown diffuse one-sided slow waves (theta- and/or delta-activity) in the symptomatic hemisphere.[83] Epileptic features such as spike-and-wave complexes have only been found in cases with simultaneous HM attacks and epileptic seizures.[75,80]

HM patients may present with confusion, impaired consciousness and fever during an attack, warranting a lumbar puncture to exclude meningitis or encephalitis. Cerebrospinal fluid can reveal pleocytosis (up to >300 leukocytes/mL) and elevated protein levels (up to >300 mg/dL) in patients in whom no evidence of a viral or bacterial infection was found.[74,84-87]

Genetics & pathophysiology of hemiplegic migraine

So far, three genes have been associated with HM (Table 3). FHM1 and SHM1 are caused by mutations in the *CACNA1A* gene located on chromosome 19p13, encoding the ion-conducting α_{1A} subunit of $Ca_V2.1$ (P/Q-type) voltage-gated Ca^{2+}-channels.[55] In response to an action potential, calcium enters the presynaptic terminal of excitatory neurons via these channels, which triggers neurotransmitter release (e.g. glutamate in the cortex) into the synaptic cleft. Approximately 25

CACNA1A missense mutations leading towards a gain-of-function of $Ca_V2.1$ Ca^{2+}-channels have been associated with FHM1 and/or SHM1.[45] The clinical spectrum of reported FHM1 families includes chronic progressive cerebellar signs, permanent cognitive deficits and impaired consciousness and seizures during HM attacks.[74,81,88] The p.Ser218Leu *CACNA1A* mutation is associated with severe attacks, consisting of cerebral oedema, seizures and coma, which may be fatal.[74,80,81,83,89,90]

Table 3: Criteria for the subtypes of hemiplegic migraine based on genetic testing and family history, according to the International Classification of Headache disorders, third edition (ICHD-3).[1]

ICHD-3 criteria
1.2.3.1 Familial hemiplegic migraine (FHM) **A.** Fulfils criteria for 1.2.3 *hemiplegic migraine* (see Table 2) **B.** At least one first- or second-degree relative has had attacks fulfilling criteria for 1.2.3 *hemiplegic migraine*. **1.2.3.1.1 Familial hemiplegic migraine 1 (FHM1)** **A.** Fulfils criteria for 1.2.3.1 *familial hemiplegic migraine* **B.** A causative mutation on the *CACNA1A* gene has been demonstrated **1.2.3.1.2 Familial hemiplegic migraine 2 (FHM2)** **A.** Fulfils criteria for 1.2.3.1 *familial hemiplegic migraine* **B.** A causative mutation on the *ATP1A2* gene has been demonstrated **1.2.3.1.3 Familial hemiplegic migraine 3 (FHM3)** **A.** Fulfils criteria for 1.2.3.1 *familial hemiplegic migraine* **B.** A causative mutation on the *SCN1A* gene has been demonstrated **1.2.3.1.4 Familial hemiplegic migraine (FHM) other loci** A. Fulfils criteria for 1.2.3.1 *familial hemiplegic migraine* B. No mutation on the *CACNA1A*, *ATP1A2* or *SCN1A* gene has been demonstrated
1.2.3.2 Sporadic hemiplegic migraine **A.** Fulfils criteria for 1.2.3 *hemiplegic migraine* **B.** No first degree or second degree relative fulfils criteria for 1.2.3 *hemiplegic migraine*

Cerebellar involvement in FHM1 and SHM1 shows similarities with two other *CACNA1A*-associated monogenic syndromes: Episodic Ataxia type 2 (EA2) and Spinocerebellar Ataxia type 6 (SCA6). EA2 is characterised by attacks of ataxia with severe vertigo, nausea, vomiting and symptoms such as dysarthria, tinnitus, dystonia, and diplopia. Patients often develop cerebellar atrophy with persistent cerebellar symptoms.[91] Approximately 50% of EA2 patients have migraine headaches and cases with episodic hemiplegia have been described in a large EA2 family.[92,93] In EA2, missense mutations and deletions leading to frameshifts have been found in *CACNA1A*.[91,94] While *CACNA1A* mutations in HM appear to result in a gain-of-function of $Ca_V2.1$ Ca^{2+}-channels, mutations in EA2 appear to lead to their loss-of-function.[55] In contrast to the point mutations in FHM1, SHM1 and EA2, SCA6 is associated with a moderate expansion of a CAG repeat stretch in exon 47 of *CACNA1A*. Patients carry 21–28 CAG triplets, compared to 4–16 in healthy individuals.[54,95] SCA6 is characterised by a slowly

progressive cerebellar ataxia, dysarthria and nystagmus. At first, symptoms may be episodic, at which stage there is marked overlap with EA2.[96,97] Migraine does not appear to be particularly prevalent in SCA6 patients, but a *CACNA1A* missense mutation was found in a family including patients with HM and progressive cerebellar ataxia, but also patients with progressive cerebellar ataxia alone.[98]

The second HM gene *ATP1A2* is located on chromosome 1q23 and encodes the α_2 subunit of a Na^+/K^+-ATPase. This pump creates a steep sodium gradient by exchanging Na^+ ions for K^+ ions, thereby facilitating removal of potassium and glutamate from the synaptic cleft into glial cells.[45] *ATP1A2* mutations found in HM are mostly missense mutations and appear to result in a loss-of-function of Na^+/K^+-ATPase function.[45,99] Additional paroxysmal neurological symptoms described in FHM2 patients include epilepsy, confusion, impaired consciousness and prolonged hemiplegia, but also permanent symptoms such as mental retardation.[45,82,83,100,101] Progressive cerebellar signs have rarely been reported in FHM2 patients.[102]

Finally, in a few HM families, missense mutations have been detected in the *SCN1A* gene that is located on chromosome 2q24 and encodes the α1 subunit of voltage-gated Na^+-channels.[103] These channels are thought to be primarily expressed on inhibitory central neurons and dysfunction of these channels is thus predicted to lead to neuronal hyperexcitability, which may facilitate HM attacks.[103–105] As mentioned, (other types of) *SCN1A* mutations are also identified in monogenic epilepsy syndromes, including Dravet syndrome (also known as severe myoclonic epilepsy of infancy (SMEI)) and generalised epilepsy with febrile seizures (GEFS+)).[56,57] The pathophysiological basis of mutations in the same gene leading to migraine versus epilepsy still remains incompletely understood.[106]

Importantly, the hypothesised common net result of all HM mutations is an increase in the cerebral levels of potassium and glutamate in the synaptic cleft, which causes an increased excitability and may explain the increased susceptibility to CSD.[45]

When a patient exhibits a typical HM phenotype, it is difficult to predict whether or not the patient has a mutation in one of the HM genes. Based on clinical symptoms during HM attacks, subtypes of HM cannot be clearly distinguished, but the presence of additional symptoms (such as chronic progressive ataxia or epilepsy) may be suggestive of certain genetic subtypes. In large FHM cohorts *CACNA1A* mutations were detected in 4-7%[107,108] and *ATP1A2* mutations in 7%.[107] Mutation detection rates in SHM vary even more, with *CACNA1A* mutations in 1–36%[109–112] and *ATP1A2* mutations in 1–

56%.[109–111] In part, these low detection rates are likely caused by confusing migraine with sensory aura with HM. Detection rates in SHM appeared to be higher in early-onset SHM, which suggests that an early age at onset may distinguish HM from common types of migraine.[111] It appears that not all individuals who carry an HM mutation develop the HM phenotype.[100,107,110] This reduced penetrance has not yet been reported in FHM3, but this may be due to the low number of FHM3 *SCN1A* mutations that have been described so far.[45]

More HM genes likely remain to be identified, as some HM patients do not have a mutation in any of the known genes. Negative test results for mutations in *CACNA1A, ATP1A2* and *SCN1A,* therefore, do not exclude the clinical diagnosis of HM. New techniques such as Next Generation Sequencing (NGS) may enable the identification of novel HM genes in the near future.[113,114]

Treatment of hemiplegic migraine

Because of the rarity of HM, large clinical drug trials have not been performed and treatment largely follows guidelines for the common forms of migraine. Preference for certain drugs is mostly based on empirical data and personal experience of the treating neurologist. There are no specific treatments for patients with specific mutations. Although the auras in HM are often more debilitating than the headache, most migraine-specific drugs for acute treatment unfortunately only affect headache.

Various trigger factors, such as stress, sleep disturbances, physical exertion, bright lights, drinks and food products have all been reported in HM.[115] However, especially stress, but possibly other trigger factors as well, are suggested to be part of the premonitory phase of the migraine attack, rather than that they represent a true trigger factor.[116] This does not apply to the triggering of attacks by (minor) head trauma, which has been convincingly reported in HM patients and is quite common.[65] Because of these reports, HM patients can be advised not to practice contact sports and for example should avoid heading balls when playing soccer. No evidence-based advice can be given about other lifestyle or dietary factors.

Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S): a vascular monogenic migraine model

One of the monogenic vascular syndromes that has been associated with migraine is Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S).[63,117] The association with migraine was first reported in a family in whom the syndrome was named hereditary vascular retinopathy (HVR).[62] In 2007 it was discovered that a collection of hereditary

neurovascular syndromes with retinal involvement and other systemic symptoms were caused by mutations in the *TREX1* gene. After this discovery, these syndromes (cerebroretinal vasculopathy (CRV), hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS) and hereditary vascular retinopathy (HRV)) were designated as Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL).[118] Heterozygous mutations in *TREX1* have been detected in sixteen RVCL families from Europe, North-America, Asia and Australia.[62,63,119–124] After summarizing the clinical symptoms found in patients worldwide, the syndrome was renamed to RVCL-S.[63] All *TREX1* mutation carriers appear to develop RVCL-S symptoms, indicating 100% penetrance.[63] The discovery of the genetic cause of RVCL-S has greatly facilitated the investigation of affected relatives within RVCL-S families, but the consecutive disease stages of RVCL-S, especially the early stages, have not been clearly delineated. RVCL-S appears to be extremely rare, but as the genetic cause has been discovered only a few years ago the condition is likely still underdiagnosed.

Clinical characteristics of RVCL-S

RVCL-S was first identified as a familial vascular retinopathy, comprising a central and peripheral microangiopathy with cotton wool spots, ischemic areas and eventually occlusion of large retinal vessels and neovascularisations, leading to haemorrhages and glaucoma. The retinopathy causes progressive loss of vision and ultimately blindness when left untreated.[119,120] Further research revealed that various cerebral and systemic conditions were also associated with RVCL-S.[62,63]

Especially in a large Dutch RVCL-S family, migraine and Raynaud's phenomenon – an abnormal vasomotor reaction in response to cold exposure – were found to be highly prevalent.[62] A genetic association study suggested an increased susceptibility to both Raynaud's phenomenon and migraine for the RVCL-S haplotype.[125] Other studies have not investigated migraine or Raynaud's phenomenon in RVCL-S in further detail, but in a large RVCL-S cohort prevalence was 59% (24/41 patients) for migraine and 40% (24/60 patients) for Raynaud's phenomenon.[63]

Focal neurological symptoms and cognitive impairment have been frequently described in RVCL-S patients.[62,119,121,126] All abnormalities on brain CT and MRI in RVCL-S patients have so far been restricted to white matter. The lesions are non-specific, but remarkable for the patients' relatively young age. Two types of lesions have been observed: 1) focal, non-enhancing T2-hyperintense lesions scattered throughout the periventricular and deep white matter; and 2) hyperintense mass lesions on T2 and hypointense lesions on T1-weighted images, enhanced with gadolinium contrast, and often surrounded by extensive oedema, displacing adjacent structures and leading to sulci

effacement.[62,121,122,126] These mass lesions are often referred to as 'pseudo-tumours' and have been reported in 75% of RVCL-S patients, most often in later stages of the disease.[63] Restricted diffusion has occasionally been observed, mainly in the centre of these lesions, but it remains unclear whether this reflects local ischemic changes or demyelination.[63] Besides major symptoms such as hemiplegia in the case of a large intracerebral mass lesion, it is currently largely unclear to what extent the intracerebral abnormalities cause which neurological symptoms in RVCL-S. Imaging in (young) *TREX1* mutation carriers without overt neurological complaints has not been performed systematically, and it is unclear whether mild intracerebral lesions may already be present in these individuals.

Although some studies still suggest the existence of systemic and non-systemic variants of RVCL-S,[127] involvement of several internal organs appears to be part of the phenotype in all RVCL-S patients. In congruence with several previous reports,[62,121,127] Stam et al.[63] found evidence of liver disease, renal disease, hypertension and anaemia (likely resulting from microscopic gastrointestinal bleeding) in the majority of examined RVCL-S patients. These findings suggest that other syndromes such as Hereditary Systemic Angiopathy (HSA), which was previously hypothesised to be different from syndromes incorporated in RVCL-S, may also actually constitute RVCL-S.[63,128] Systematic and detailed measurements are needed to further clarify the occurrence and severity of internal organ dysfunction in different stages of RVCL-S.

Life expectancy is decreased in RVCL-S patients, with an average age at death of 53 years (range 32–72 years).[63] While internal organ involvement may limit RVCL-S patients' life expectancy in some cases, neurological deterioration appears to be the primary cause of this decreased life expectancy, as in most described cases the cause of death was a pneumonia or sepsis in the setting of general debilitation.[63]

Genetics & pathophysiology of RVCL-S

TREX1 is the most abundant 3'-5' exonuclease in mammalian cells. Normal TREX1 protein resides in the cytoplasm in a protein complex attached to the endoplasmic reticulum membrane and likely translocates across the cell to clear products of DNA repair, replication and retrotranscription.[129,130] The *TREX1* gene consists of a catalytic domain, involved in enzymatic activity, and a carboxyl terminal domain, probably influencing the localization of the TREX1 protein.[118] All five known *TREX1* mutations in RVCL-S are located in the carboxyl terminus of TREX1 and result in frameshifts leading to truncated TREX1 protein that lacks part of the carboxyl terminus,[63] which hampers binding of TREX1 to the endoplasmic reticulum.[124] While mutant TREX1 retains its exonuclease activity, it is

thought to lack proper subcellular context and to be located throughout the nucleus and cytoplasm.[124]

TREX1 mutations have not only been found in RVCL-S, but also in Aicardi-Goutières Syndrome (AGS),[131,132] Familial Chilblain Lupus (FCL),[133,134] and (neuropsychiatric) Systemic Lupus Erythematosus (SLE).[135,136] Mutations in FCL and AGS are primarily found in the catalytic domain of TREX1, while mutations in RVCL-S and SLE are most often found in the carboxyl terminus. In AGS, FCL and SLE several types of mutations have been reported, including missense and frameshift mutations.[137] Some of these frameshift mutations are located closely to the mutations found in RVCL-S, making frameshift mutations in the carboxyl terminus of TREX1 not unique to RVCL-S.[138] The exact functional effects of these different types of mutations on these different locations of TREX1, however, remains an enigma.

As AGS, FCL and SLE are all associated with a disruption of type I interferon (IFN) metabolism, this has incited research into the link between TREX1 and (auto)immunity. Deficiency of TREX1 is postulated to cause accumulation of intracellular nucleic acids, which may initiate an IFN-α-mediated innate immune response leading to inflammation and autoimmunity.[139–141] A possible example of this phenomenon is that *Trex1* deficient mice were shown to develop inflammatory myocarditis.[142] More importantly, in AGS patients expression of certain interferon-stimulated genes was found to be elevated in serum and cerebrospinal fluid.[138,143] In addition, a substantial increase in the release of proinflammatory cytokines (IL-6) and chemokines (CXCL10 and CCL5) was detected.[143] These results provide further confirmation that interferon activity is an important part of the pathophysiology of this *TREX1*-related disease. A similar interferon signature was described in a case report of an RVCL-S patient, albeit using slightly different methods.[144] Another recent study found that the carboxyl terminus of TREX1 is important in the regulation of oligosaccharyltransferase activity in the endoplasmic reticulum, and thereby in preventing glycan and glycosylation defects that can lead to immune disorders.[145]

Clinical features of RVCL-S such as the vascular retinopathy, Raynaud's phenomenon, brain white matter lesions, kidney and liver dysfunction and migraine point towards systemic involvement of small vessels and more specifically of the endothelium. Activation of the endothelium results in a proinflammatory, procoagulatory, and proliferative milieu.[146] Both impaired endothelial-dependent vasodilatation and a mismatch between endothelium-derived vasoconstrictors (primarily endothelin-1) and vasodilators (primarily nitric oxide and prostacyclin) were demonstrated in Raynaud's phenomenon.[147] Similar mechanisms may be involved in RVCL-S. A recent study demonstrated

endothelial dysfunction in RVCL-S patients and impaired endothelial independent vasoreactivity of dermal microvasculature in CADASIL patients.[148] Both disorders seem to have an increased vascular stiffness. Ultrastructural pathological studies in tissue from RVCL-S patients revealed thicker endothelial cells with increased vesicles and coarse cytoplasm, and thicker, multi-laminated basement membranes of endothelial cells.[63,149] Another interesting finding in this respect is the reported increase in circulating levels of Von Willebrand Factor in a patient with Hereditary Systemic Angiopathy, which may in fact be RVCL-S.[128] Von Willebrand Factor is stored in Weibel-Palade bodies in the endothelial cells and is considered one of the best circulating markers of endothelial dysfunction, and was also found to be elevated in migraine patients.[32,146,150] Another circulating endothelial marker that is of interest for RVCL-S research is angiopoietin-2 (Ang-2), which is also stored in Weibel-Palade bodies.[151] Ang-2 is released after endothelial activation and causes destabilised endothelium, vascular leakage and abnormal microvascular remodeling.[152–156] Ang-2 has not yet been investigated in RVCL-S.

The postulated involvement of endothelial dysfunction may turn out to be an important feature to propose RVCL-S as a vascular model for migraine and (cerebral) small-vessel diseases that are an important cause of cognitive impairment in the elderly population.[157] By unravelling the hypothesised endothelial dysfunction in RVCL-S new pathophysiological mechanisms of both migraine and small-vessel disease may be revealed.

Treatment of RVCL-S

A treatment to cure RVCL-S is not available, but some symptomatic treatments can be tried. The common policy for migraine treatment can be followed, and progression of the retinopathy can be halted by laser photocoagulation of aberrant retinal vasculature. As the occurrence and severity of internal organ dysfunction remains to be further specified, it is unclear in what proportion of RVCL-S patients treatment is needed for these symptoms. In patients with a ‘pseudo-tumour’ physicians are tempted to try different treatments, as a patient may show sudden and progressive significant neurological deficits. There is no evidence for an effect of immunosuppressive treatment on the underlying lesions, although surrounding oedema may decrease. However, it is important to note that spontaneous regression of the intracerebral lesions has been described to occur over the course of several months.[126] More knowledge of the pathophysiology of RVCL-S is thus indispensable to be able to find safe and effective treatments.

Outline of the thesis

In this thesis the clinical phenotypes and pathophysiology of rare monogenic and common complex forms of migraine are investigated to ultimately identify novel treatment targets for these disorders. The studies described can be divided into two main parts. **Part I** describes studies on hemiplegic migraine (HM), a neuronal and glial monogenic model of migraine, in which we investigated treatment options for hemiplegic migraine and aimed to complete the clinical and genetic spectrum of hemiplegic migraine. **Part II** of this thesis describes studies focussing on Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S), a vascular monogenic model of migraine. To identify the consecutive clinical stages of RVCL-S and to identify biomarkers for the disease, an observational cross-sectional case-control study was performed in the Dutch RVCL-S families: the RVCL-ID study.

Part I: Hemiplegic Migraine – a neuronal and glial monogenic migraine model

Chapter 2 describes a review of the literature on diagnostic procedures and treatment options for hemiplegic migraine, aiming to provide a guideline for physicians treating hemiplegic migraine patients despite the lack of large drug trials. In **chapter 3** a hemiplegic migraine family is described in which efficacy of prophylactic treatment with sodium valproate and lamotrigine was observed. In **chapter 4** we describe the long-term follow-up of a hemiplegic migraine family with a novel *ATP1A2* mutation, illustrating the severe end of the phenotypic spectrum of hemiplegic migraine, which is often not recognised as migrainous in clinical practice. **Chapter 5** describes the discovery of novel *SCN1A* mutations in two families with pure hemiplegic migraine. *SCN1A* mutations are still quite rarely identified in hemiplegic migraine and the distinction between the pathophysiological mechanisms behind these mutations and other *SCN1A* mutations that cause epilepsy syndromes remains to be elucidated. **Chapter 6** contains a critical analysis of the proposed association of the *PRRT2* gene and hemiplegic migraine, which appears to be different from the associations observed with the well-known hemiplegic migraine genes, *CACNA1A*, *ATP1A2* and *SCN1A.* In addition, a previously proposed association of benign familial infantile convulsions and a mutation in the *ATP1A2* gene is questioned. In **chapter 7** a clinical comparison of hemiplegic migraine patients with and without a confirmed mutation in *CACNA1A*, *ATP1A2* or *SCN1A* is described, investigating whether there may be a phenotypical spectrum within hemiplegic migraine as it is currently defined in the International Classification of Headache Disorders. This phenotypic spectrum is suggested to also harbour different genetic backgrounds and it is proposed that hemiplegic migraine, in part, may also be caused by complex genetic mechanisms. Finally, a whole exome sequencing study in hemiplegic migraine is described, in which we aimed to identify novel genes for hemiplegic migraine.

Part II: Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations – a vascular monogenic migraine model

Chapter 8 describes the identification and detailed description of the comprehensive phenotype of Dutch RVCL-S patients of different ages, including neurological and internal organ involvement. In **chapter 9** a pathophysiological study on RVCL-S is described, in which samples collected for the RVCL-ID study were utilised to measure circulating markers of endothelial function, including Von Willebrand factor and angiopoietin-2. **Chapter 10** describes the attempt to investigate the role of *TREX1* mutations in cerebral vascular disorders without clear diagnosis, but with an appearance similar to Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), a small-vessel disease resembling RVCL-S.

Finally, **chapter 11** provides a general discussion of the thesis, reviewing the results and discussing future possibilities for research of monogenic models of migraine to increase our knowledge of migraine pathophysiology.

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