Stroke and migraine: Translational studies into a complex relationship

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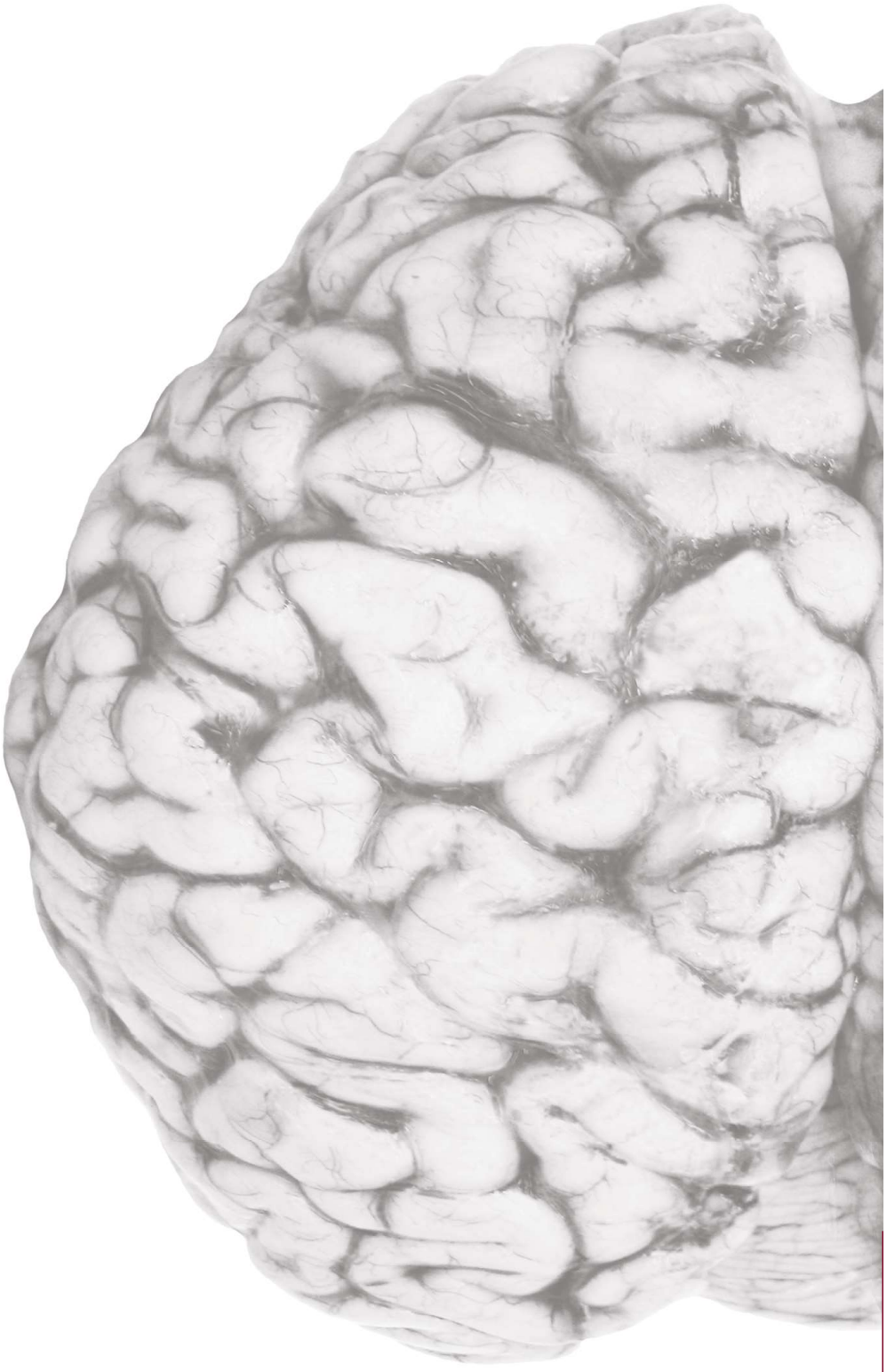

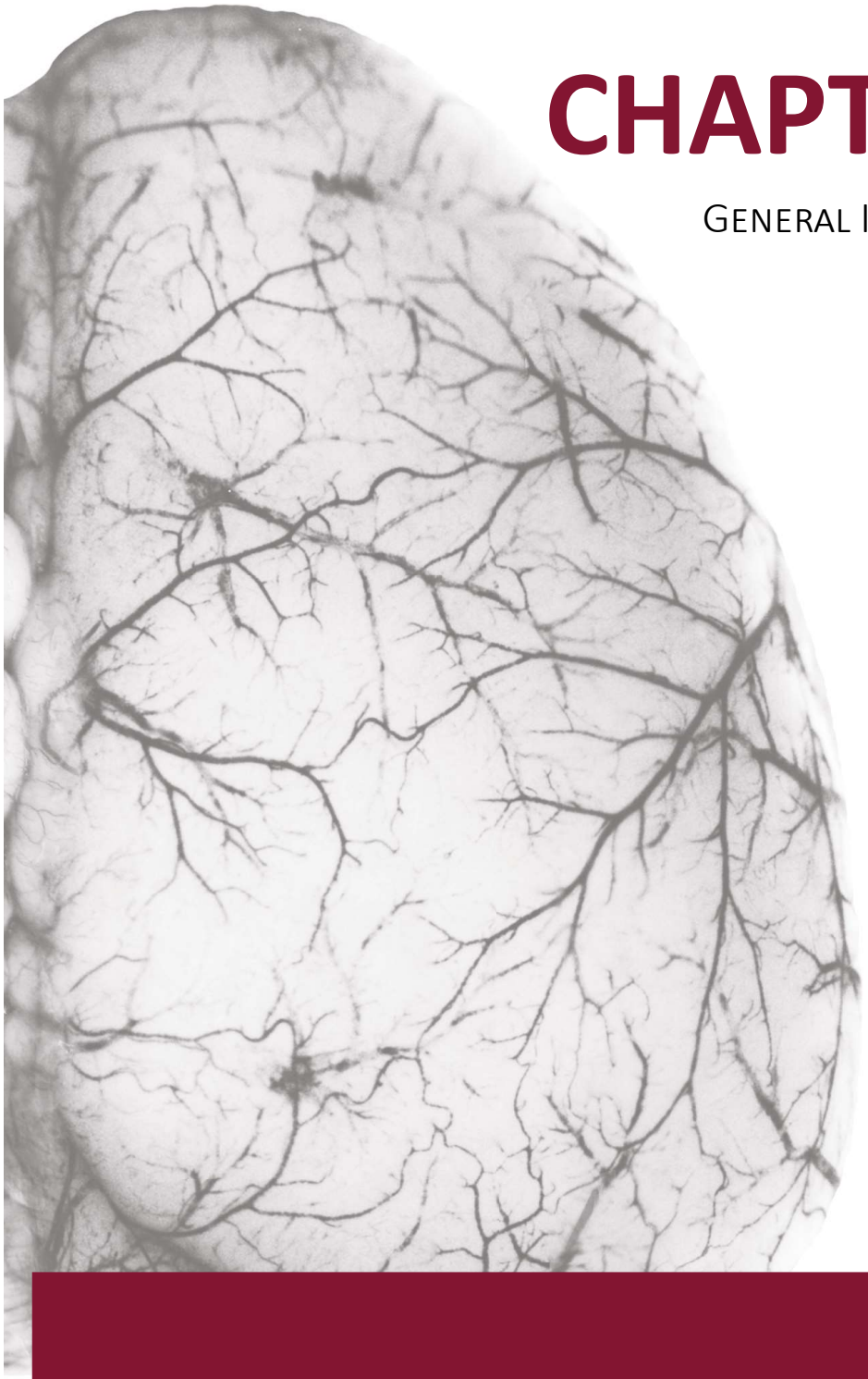

CHAPTER 1

General Introduction

1

GENERAL INTRODUCTION

Although stroke and migraine are generally considered to be two very different disease entities, they actually are closely connected and their pathophysiological overlap becomes increasingly clear. In this thesis we investigate, in a translational manner in patients and experimental animal models, the relationship between migraine and ischemic stroke and between migraine and delayed cerebral ischemia after subarachnoid hemorrhage.

1. Epidemiology of stroke and migraine

1.1 Ischemic stroke

Stroke is the second most frequent cause of death and the most frequent cause of disability worldwide,[1] with a global incidence of more than 10 million and a prevalence of almost 26 million.[2] In the Netherlands, every year approximately 46,000 people get a stroke. According to 'The Stroke Council of the American Heart Association / American Stroke Association' criteria, the definition of stroke is "an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction".[3] Roughly, there are two main stroke subtypes: ischemic stroke and hemorrhagic stroke. Ischemic stroke is the most common (about 80%) type of stroke. Although hemorrhage stroke is less common its long term consequences are often severe and therefore both subtypes have great impact on a patient's daily life.

Ischemic stroke occurs when blood flow to the brain is restricted due to occlusion of a cerebral artery, typically by a local thrombus or an embolus (Figure 1). The global incidence is almost 7 million with a prevalence of 18 million.[2] In the Netherlands, the incidence is almost 20,000

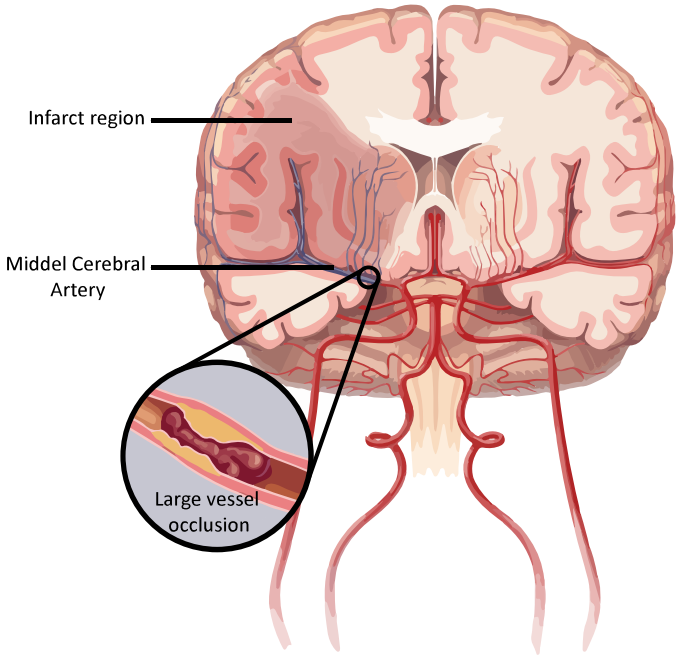

Figure 1. Schematic illustration of an ischemic stroke (modified from strokecenter.org)

per year.[4] Different subtypes are described, according to their underlying cause, which affects stroke management. The 'Trial of Org 10172 in Acute Stroke Treatment' (TOAST) classification[5] describes five subtypes: (I) large-artery atherosclerosis (embolus / thrombosis), (II) cardioembolism, (III) small-vessel occlusion (lacunar infarct), (IV) stroke of other determined etiology, and (V) stroke of undetermined etiology. There is a remarkable gender difference in stroke with men having an overall higher risk for first-ever stroke at medium age,[6] where women have a higher risk in young (< 55 year) and older (> 75 year) ages.[7] Also, the prevalence and average age of first-ever stroke is higher in women.[6,7] Women have a higher burden after stroke, with more often physical impairment and depression than men.[7]

1.2 Migraine

Migraine is a common episodic brain disorder affecting approximately 15% of the population.[8] Migraine is characterized by attacks of severe, usually throbbing unilateral headache that are accompanied by nausea, vomiting, photo-, and / or phonophobia.[9] Attacks typically last 4 to 72 hours. Because of its high prevalence and major social and economic burden migraine was rated one of the most disabling common chronic neurological disorders.[10] Two main types of migraine can be distinguished: migraine without aura and migraine with aura.[9] The latter is present in about one third of patients and is characterized by an aura that can precede the headache. An aura consists of transient focal neurological symptoms affecting mainly the visual system but that can also include sensory, aphasic and motor symptoms. Migraine is a heterogenic disease with an attack frequency that can vary between and within patients from a few attacks per year up to a few per week; also the same patient can suffer from migraine with and without aura attacks. Migraine affects more women than men in a 3:1 ratio.[9,11]

1.3 The stroke-migraine connection

Evidence is accumulating that migraine, especially migraine with aura, is an independent risk factor for ischemic stroke,[11-15] especially in women. At first this seems unexpected given the clinical disease characteristics that are quite different between both disorders, including the sex difference. Whereas migraine is a chronic disorder most common in young to middle-aged women (age 25 to 55 years), stroke is an acute event that typically occurs in middle aged men. Regardless, additional clinical evidence for the co-morbidity of stroke and migraine comes from: (I) the possible existence of migraineous infarctions,[16,17] (II) the co-occurrence of migraine and cervical artery dissection,[18] (III) shared risk factors like hypercoagulability[19] and endothelial dysfunction,[20] (IV) the fact that certain drugs to treat migraine, such as triptans and ergotamines, have been associated with increased stroke risk,[21] and (V) genetic evidence linking stroke and migraine in multiple monogenic diseases.[22,23]

2. Primary and secondary ischemic damage in stroke; core and penumbra

Multiple complex mechanisms are responsible for infarct maturation during and after vessel occlusion. Although the exact mechanisms are still largely unknown, a typical temporal pattern seems to occur after a focal perfusion deficit.[24] Within this temporal and spatial continuum the infarct territory can be divided into two main areas: the ischemic core and the penumbra, or 'tissue at risk' (Figure 2).[25,26] Within minutes after the ischemic event, cells contributing to the core become necrotic with membrane breakdown, dysfunctional cellular metabolism and energy supply, disturbed ion homeostasis, and loss of cell integrity. The tissue surrounding the core, however, is 'struggling to survive' due to collateral blood supply being borderline

sufficient. Cells in the penumbra are metabolically active for some time, until the disruption of the cellular homeostasis in these cells also leads to cell death. It is increasingly clear that inflammatory factors[27] and blood-brain-barrier (BBB) breakdown[28] play an important role in the transition of penumbra tissue into core tissue. The transition process can take up to several hours, which has direct impact on the 'time-to-treat' window of stroke patients. The first destructive cascade that is activated after a perfusion deficit is cellular excitotoxicity, which contributes to a large extent to the tissue damage. Excitotoxicity includes the production of reactive oxygen and nitrogen species (ROS and RNS) and acidosis. Within minutes, and lasting up to several hours, multiple pathophysiological events take place: (I) a rise of extracellular K^+, (II) presynaptic terminal depolarization, (III) excessive extracellular neurotransmitter accumulation, (IV) N-methyl-D-aspartate (NMDA)-receptor activation, (V) loss of ion homeostasis (Ca^{2+}, K^+, Na^+, H^+, Cl^-, HCO_3^-), and (VI) a rise of neuronal and glial intracellular Ca^{2+} resulting in cytotoxic edema.[29-31] Secondary mechanisms contributing to increased tissue damage are BBB breakdown, reperfusion injury, inflammation and apoptosis.[32] Protective and regenerative mechanisms to prevent and repair damage of a stroke also occur actively in the peri-acute and chronic phase after ischemic onset.[32] However, the molecular pathways involved in these events are still to be unraveled. Investigating at the molecular level, by elucidating the peptides, (amino-) metabolites and lipids that show changes in the various, especially the early, stages of a stroke can help us to dissect the pathophysiology of stroke and can eventually lead to new therapeutic targets to treat patients.

3. The role of spreading depolarization and neurovascular coupling in the shared pathophysiology between stroke and migraine

3.1 Spreading depolarization

Spreading depolarization (SD) is the generic term for a self-propagating wave of membrane depolarization in neuronal and glial cells which travels through cerebral grey matter of the central nervous system and which is accompanied by a period of electrical silencing. These depolarization waves have been described in humans in ischemic stroke,[33] in subarachnoid hemorrhage (SAH)[34] and in traumatic brain injuries[35] at the site of the injury where they are referred to as anoxic depolarizations (ADs) and peri-infarct depolarizations (PIDs). SDs are also considered to be the underlying pathophysiological mechanism of a migraine aura.[36,37] In migraine aura a SD that originates in the visual cortex and spreads to frontal cortical regions is referred to as cortical spreading depression (CSD), named after the neuronal depression that follows the sharp wave front of hyperexcitation (with concomitant neuronal and glial cell depolarization). SDs can therefore be seen as a spectrum that includes PID, AD and CSD depending on the disease type and is also referred to as the stroke-migraine depolarization continuum.[24,38]

3.1.1 Spreading depolarization in ischemic stroke

In a pathological condition, for example during ischemic stroke onset, depression of spontaneous neuronal activity is seen 10-20 seconds after a blood flow reduction below a certain threshold (15- 23 mL / 100g / min).[25] Neurobiological mechanisms that are active in this initial period are neuronal hyperpolarization, loss of synaptic activity, reduction of vesicular transmitter release by adenosine mediation, and reduced energy consumption (that acts as a survival mechanism of the tissue to cope with the ischemia). Within 2-5 minutes, AD occurs resulting in an even further reduced blood flow (5-10 mL / 100 g / min) and depression of

activity.[38,39] The AD originates from the core, and spreads via the penumbra into healthy tissue. This wave is triggered by loss of membrane integrity due to hypoxia and energy depletion. Notably, after this first depolarization wave, multiple PID waves erupt from the penumbra, spreading into the penumbra, core and healthy tissue. Due to the energy mismatch created by these PIDs, each wave will turn part of the penumbra tissue into a permanently depolarized and necrotic state,[40-42] and therefore co-determines the severity of stroke outcome.

3.1.2 Spreading depolarization in migraine

Under normal conditions, neurons and their dendrites have a membrane potential that enables them to fire action potentials, which is the way neurons communicate. This membrane potential is maintained by active ion pumps. During CSD in a migraine aura, this homeostasis is disrupted, resulting in: (I) a near-complete breakdown of ion gradients,[43] (II) increased extracellular K^+ level, (III) loss of electrical activity,[44] (IV) swelling of neurons,[45] (V) sustained

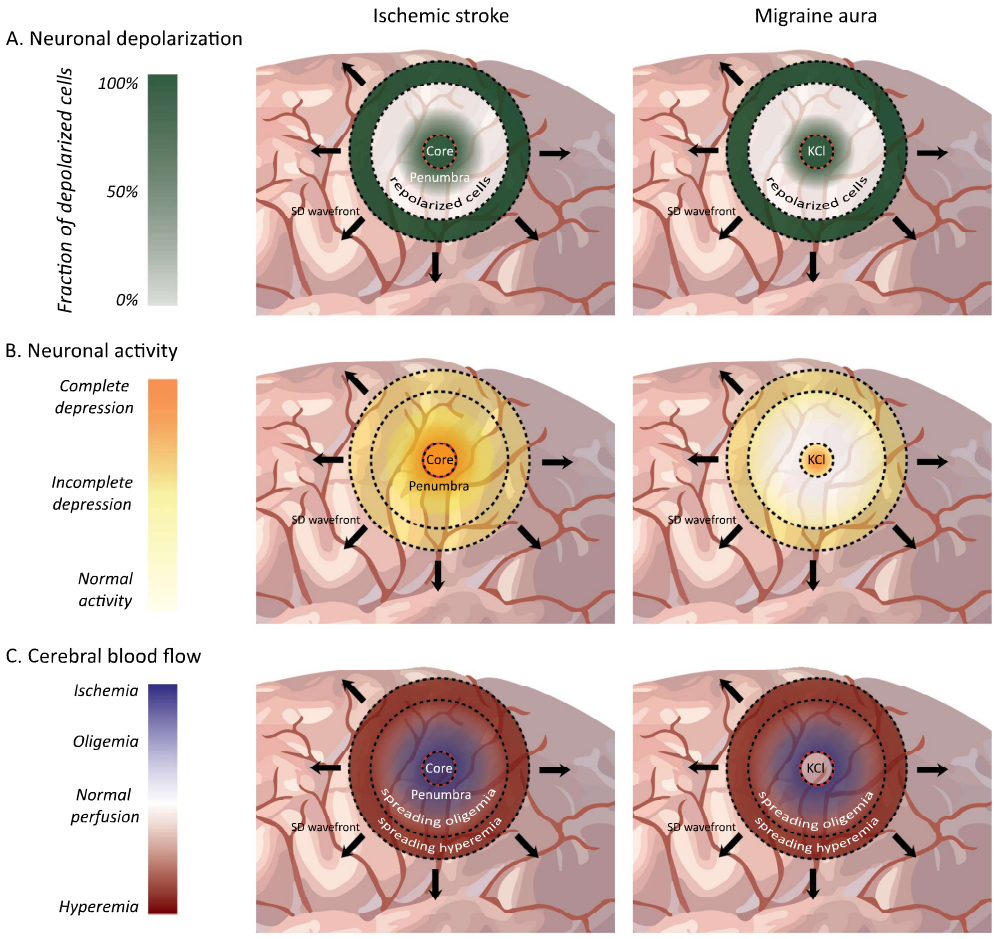

Figure 2: Schematic illustration of SD waves triggered by occlusion of a cerebral vessel (left) as seen during ischemia and triggered by high potassium (right) occurring during a migraine aura, with (A) neuronal depolarization, (B) Neuronal activity and (C) Cerebral blood flow. (Modified from Dreier *et al.* 2015[24] with permission). SD – Spreading Depolarization.

depolarization,[46] and (VI) a hemodynamic response.[47] Unlike SD events in stroke, CSDs in migraine are considered rather benign transient disturbances of (cortical) brain function without permanent damage. Whether SD also occurs in migraine without aura, also referred to as 'silent aura', is debated.[48,49]

3.2 Neurovascular coupling

Vessels are a major player in the pathology of stroke and likely also in migraine, in the first place due to their involvement in neurovascular coupling. As a result of neurovascular coupling, a hyperemic response occurs to meet the increased energy demand when PIDs circle around the ischemic core (in the case of stroke) or when a CSD wave spreads through the cortex (in the case of a migraine aura).[50] Briefly before and prolonged after this phenomenon, hyperemia and oligemia are present.[51] In pathological (ischemic) tissue, AD and PIDs are accompanied with paradoxical vasoconstriction resulting in oligemia.[52,53] The AD / PIDs and additional decrease in resting cerebral blood flow (rCBF) is also called spreading ischemia,[54] due to inverse neurovascular coupling (Figure 2).[52] In such pathological tissue, the hypoperfusion wave travels, in contrast with CSD, together with the spreading depolarization at the same time through the tissue, where it enters the vicious cycle of an inverse hemodynamic response and energy supply / demand mismatch. The trigger for PIDs is the massive misbalance in ion homeostasis that induces a vicious circle of ischemia, depolarization and vasoconstriction with an increased infarct territory as the devastating result.[39,55] PIDs under ischemic conditions are seen in numerous experimental animal models.[56-58] CSD is thought to occur in patients that have migraine with aura, although the most convincing evidence thus far in humans came from analyzing indirect vascular responses seen with imaging techniques[37] and correlations with clinical characteristics,[59] adding to the debate on how relevant CSD is in humans.[60] In contrast, CSD has been studied widely in animal models in which it has been shown that it indeed is the likely cause of the aura.[61,62]

3.3 Vascular dysfunction

The connection of stroke and migraine has also been attributed to multiple vascular pathologies, such as endothelial dysfunction and coagulation abnormalities.[63] Endothelial dysfunction includes reduced vasodilatation, increased endothelial derived vasoconstriction (vasospasm) and subsequent impairment of cerebral vascular reactivity. These processes can subsequently lead to an increase in coagulation factors, increased release of inflammatory factors that eventually can lead to atherosclerosis and increased stroke risk. Coagulation abnormalities (primarily or secondarily due to endothelial dysfunction) are found in stroke[64,65] as well as in migraine[66-68] patients and include increased platelet-activating factor (PAF), increased VonWillebrand Factor (VWF), both of which are released by or triggered by endothelial cells.

4. Monogenic disorders in stroke and migraine

There are a number of monogenic diseases in which ischemic stroke and migraine are part of the clinical spectrum.[69] Understanding the genetics and molecular mechanisms of these diseases provides an unique opportunity to further unravel the pathophysiology of the stroke-migraine association. Here three monogenic diseases will be discussed: (I) Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL),[70] (II) Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S),[71] and (III) Familial Hemiplegic Migraine (FHM).[72] Both CADASIL and RVCL-S belong to

the group of small vessel diseases;[69] a condition in which the walls of small arteries in the brain are damaged.[73] In contrast, FHM is considered more a disease of neurologic than of vascular dysfunction.

4.1 CADASIL

CADASIL is the most common type of hereditary small vessel disease and characterized by progressive development of subcortical infarcts, starting at middle age[74-77] with cognitive decline even before first stroke onset.[78,79] Remarkably, approximately 40% of CADASIL patients suffer from migraine with aura,[80] which in many is the first presenting symptom, sometimes decades before the onset of other disease characteristics. As the disease progresses, accumulation of lacunar infarcts, microbleeds and brain atrophy result eventually in severe vascular dementia. CADASIL is caused by mutations in the ***NOTCH3*** gene, which encodes the NOTCH3 protein that is mainly expressed in vascular smooth muscle cells.[81] CADASIL mutations typically alter the number of cysteines that are responsible for correct folding of the protein's extracellular domain ($NOTCH3^{ECD}$).[82,83] Misfolding eventually leads to accumulation of mutant protein in vascular smooth muscle cells (VSMC),[77,84] degeneration of these cells, vessel wall thickening, and the occurrence of dense deposits of granular osmophilic material (GOM) in the vessel wall. Typically the abnormalities are observed in small- and medium-sized arteries.[85] Various transgenic mouse models are available that express NOTCH3 protein with CADASIL mutations, either from a human cDNA overexpression construct,[86-88] a rat[89] or human[90] genomic overexpression construct, or a mouse knock-in construct.[91,92] To more or lesser extent, these animal models exhibit key features of the disease.[90,93] However, brain imaging abnormalities seen in CADASIL patients, have not yet been found in these mice.

4.2 RVCL-S

RVCL-S is a systemic small vessel disease with prominent vasculopathy of, most profoundly, retina, brain and kidney that may lead to visual loss, cognitive disturbances, depression and kidney dysfunction, which starts at middle-age.[71,94-96] About half of RVCL-S patients also suffer from migraine (with or without aura), as became clear from investigating all 11 known RVCL-S families in the world.[71] RVCL-S patients also have an increased ischemic stroke risk as evidenced by the small white matter infarcts seen in many patients.[71] RVCL-S is caused by heterozygous C-terminal frameshift mutations in the *TREX1* gene,[95] which encodes the major mammalian 3' - 5'exonuclease that has multiple possible functions such as acting as cytosolic DNA sensor to prevent autoimmunity.[97,98] A study of mutant cells and a transgenic mouse model that expresses a *TREX1* mutation pointed at an aberrant release of free glycans due to abnormal oligosaccharyltransferase (OST) function as a possible mechanism for the vasculopathy.[99]

4.3. FHM

FHM is a monogenic subtype of migraine with aura and evidence is accumulating that it is linked to stroke.[9] FHM is characterized by long-lasting hemiparesis during the aura phase,[9] with headache features[100] and trigger factors[101] that are similar to those in common migraine with aura. Three genes, FHM1 to FHM3, have been identified that all encode ion transporters.[72] FHM1, the gene that is most prominently linked to stroke, is caused by certain missense mutations in the *CACNA1A* gene,[102] which encodes the α1A subunit of voltage-gated $Ca_V2.1$ (P / Q-type) calcium channels. These channels are located at most, if not all, synaptic terminals of the central nervous system where they regulate neurotransmitter release.[103,104] FHM1

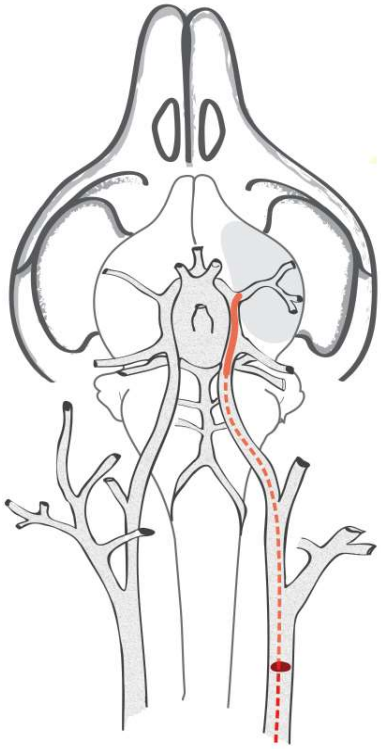

Figure 3. Experimental transient intraluminal suture model for middle cerebral artery occlusion (MCAO) in mice.

mutations cause a left-shift in the action voltage, prolongation of opening of $Ca_V2.1$ channels, and increased neurotransmitter release. In the cortex, this results in an enhanced glutamate release that explains the increased SD sensitivity seen in transgenic mice that express $Ca_V2.1$ channels with FHM1 mutations.[105-109] These transgenic mice also were shown to be a relevant model to study the relation of stroke and migraine.[56,110]

5. Techniques to investigate the relation between stroke and migraine

5.1 Experimental stroke model in mice

Various cerebral stroke models are described in literature, ranging from global (transient whole circulatory arrest) to focal (transient or permanent occlusion of a cerebral artery) occlusion of cerebral blood flow. These models give us the opportunity to study stroke-induced mechanisms with the final goal of reducing patient burden after an infarct.[111] One of the most common causes of ischemic stroke seen in patients is the occlusion of the middle cerebral artery (MCA) by a thrombus or embolus.[112] This stroke subtype is best mimicked by the experimental middle cerebral artery occlusion model (MCAO) with reperfusion, which therefore, is the most widely used model in experimental stroke research (Figure 3). With this model, the MCA is occluded by the temporary introduction of a filament into the intracerebral artery (ICA) that is maneuvered towards the origin of the MCA where it blocks blood flow. The MCAO model allows for ischemic core and penumbra development, of which the ratio and severity is directly dependent on the occlusion time. Occlusion of the MCA for 30-60

minutes will make the lateral striatum (caudoputamen) ischemic with or without ischemia of the frontoparietal cortical region. Advantages of this model over other models, such as distal transient / permanent MCAO, is that it is minimally invasive concerning the research target area (the brain), since skull integrity is maintained and the occlusion is more stable compared to for instance embolic stroke models.[113] Therefore, MCAO reduces the amount of confounding factors of massive surgery and thus mimics the clinical situation as accurate as possible.

5.2 State-of-the-art imaging techniques in mice

5.2.1 Magnetic Resonance Imaging

Using magnetic resonance imaging (MRI) as a readout technique for infarct characteristics, avoids disadvantages such as: (I) histological validation (the current golden standard) that introduces errors as there will be changes in brain morphology from processing brain sections (swelling / shrinkage of tissue), (II) the manual-labor-intensive nature of infarct volume analysis, and (III) the necessity to sacrifice the animal making longitudinal studies and multiple readout times unfeasible. Anatomical spin-spin relaxation time contrast T2 MRI sequence can detect ischemic lesions in a way that they can be analyzed in a longitudinal manner.[114-116] This T2 sequence is shown to be sensitive to vasogenic edema which is one of the mechanisms active during infarct development.[117] In clinical research, multiple algorithms for automatic detection, segmentation and classification of stroke areas in the brain have been developed.[118] However, segmentation of brain lesion in mouse MRI data still heavily relies on manual time-consuming protocols.[119]

5.2.2 Mass Spectrometry Imaging

To simultaneously analyze the distribution of hundreds of molecules from a tissue sample[120] within its histological context,[121] mass spectrometry imaging (MSI) can be used.[122] MSI can distinguish molecules from different classes such as peptides, (amino-) metabolites, proteins and lipids. The identity of molecules is determined using their unique mass-to-charge ratio (m/z). Matrix-Assisted-Laser-Desorption / Ionization (MALDI) MSI is a method to ionize molecules in the target tissue. MSI involves matrix deposition onto a tissue section, where after a laser beam allows desorption and ionization of molecules that subsequently are detected by the mass analyzer. From this data, 2D images are reconstructed that provide detailed information on the spatial distribution of the respective metabolites. To avoid confounding distortion of sections from different samples by the various procedures (e.g. cutting, processing), 2D MSI images can be co-registered with for example histological images, MRI images or brain atlases.[123,124] Arguably, tissue preparation is the most important factor determining the success of a MSI experiment, especially for molecular classes that are highly susceptible to *post mortem* changes, foremost ATP and ADP,[120,121,125,126] that are important to evaluate molecular mechanisms relevant to stroke. Multiple tissue preparation methods have been reported that have their advantages and limitations concerning different molecular classes,[120] but at present none of them is ideal.

5.3 State-of-the-art CT techniques in patients

Initial triage and management in ischemic stroke is crucial in patients who come to the emergency room with signs and symptoms of acute ischemic stroke, since time to reperfusion is highly important for the outcome of the patient. Along with the neurologic exam, radiological imaging is eminent for diagnostic and therapeutic purposes. In today's

clinic, a CT-scan is made to visualize the possible infarct territory. Non-contrast CT (NCCT) is used to differentiate between ischemic and hemorrhagic stroke, and to exclude other possible causes for the presenting symptoms, such as a subdural hematoma. Additionally, CT angiography (CTA) is increasingly performed, which gives important information concerning the presence and location of a thrombus and functional collateral and anastomotic function, which is crucial information for mechanical thrombectomy management.[127,128] Upcoming is the opportunity for CT perfusion (CTP) in acute stroke management. This relatively new technique can provide additional information on the viability of the infarcted tissue. CTP includes information concerning tissue perfusion, such as cerebral blood volume and flow (CBV and CBF, respectively), mean transit time (MTT), time-to-peak (TTP) and blood-brain-barrier permeability (BBBP).[129-132]

6. Scope and outline of the thesis

In this thesis, studies of experimental ischemic infarct rodent models and results from epidemiological human studies investigating ischemic stroke patients are combined to investigate relevant mechanisms that (possibly) underlie migraine and stroke. Understanding the molecular mechanisms underlying this comorbidity will eventually help us to identify possible therapeutic targets to reduce infarct size and improve clinical outcome.

Part I of the thesis describes advances in the methodology to obtain and analyze infarct data of experimental stroke in multiple monogenetic stroke and migraine mouse models. Chapter 2 describes a renewed sacrificing method, which is now used for mouse tissue collection after experimental stroke in order to reduce post-mortem molecular degradation as much as possible. This method is applied in Chapter 3 to investigate, with state-of-the-art MALDI-MSI techniques, brain tissue of transgenic mice with an FHM1 missense mutation in the CACNA1A gene that underwent experimental MCAO. Lipids are analyzed with respect to the core and penumbra at different time points after experimental infarct induction in order to find potential altered molecular pathways in these infarct areas which might be responsible for infarct enlargement and maturation. In Chapter 4 an automated method for MRI lesion segmentation in mice is developed to overcome current obstacles of tedious manual segmentation that, in principle, is error-prone. The segmentation tool is used for data analysis in Chapters 5 and 6. In Chapter 5 the tool is used to investigate infarct volume, in addition to parameters of vascular functionality, in transgenic mice with a human RVCL-S mutation to investigate whether, and to what extent, these mice show vascular dysfunction seen in patients with RVCL-S. In Chapter 6 transgenic RVCL-S, CADASIL, and FHM1 mice are investigated and compared, aimed to identify possible stroke vulnerability changes in these animal models, as seen in patients with the same mutation. Here we also included neuronal hyper-excitability experiments by examining CSD characteristics as possible mechanism for stroke vulnerability.

Part II describes data of clinical studies in which state-of-the-art CT techniques are used to detect radiological infarct characteristics in patients with migraine or headache and stroke. In Chapter 7 we used modern CTA and CTP techniques to investigate whether radiologic stroke features and occurrence of secondary brain damage differed in stroke patients with and without migraine and whether this resulted in different outcomes after intravenous-thrombolysis and / or thrombectomy. In Chapter 8 we investigated the association between migraine and cerebrovascular atherosclerosis in patients with acute ischemic stroke. A general discussion about the interpretation of the experimental and clinical studies and suggestions

for future research is presented in Chapter 9.

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