Stroke and migraine: Translational studies into a complex relationship

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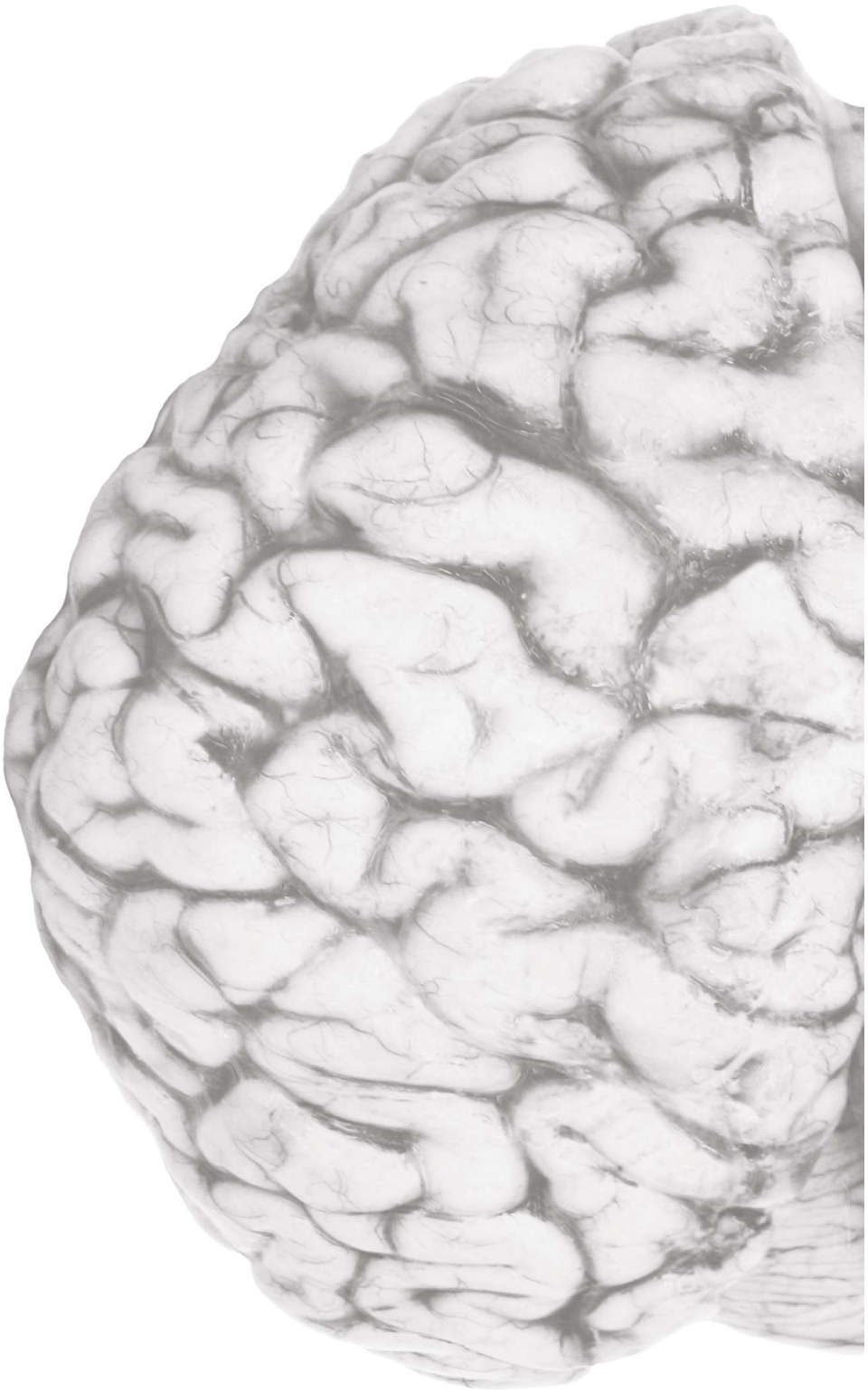

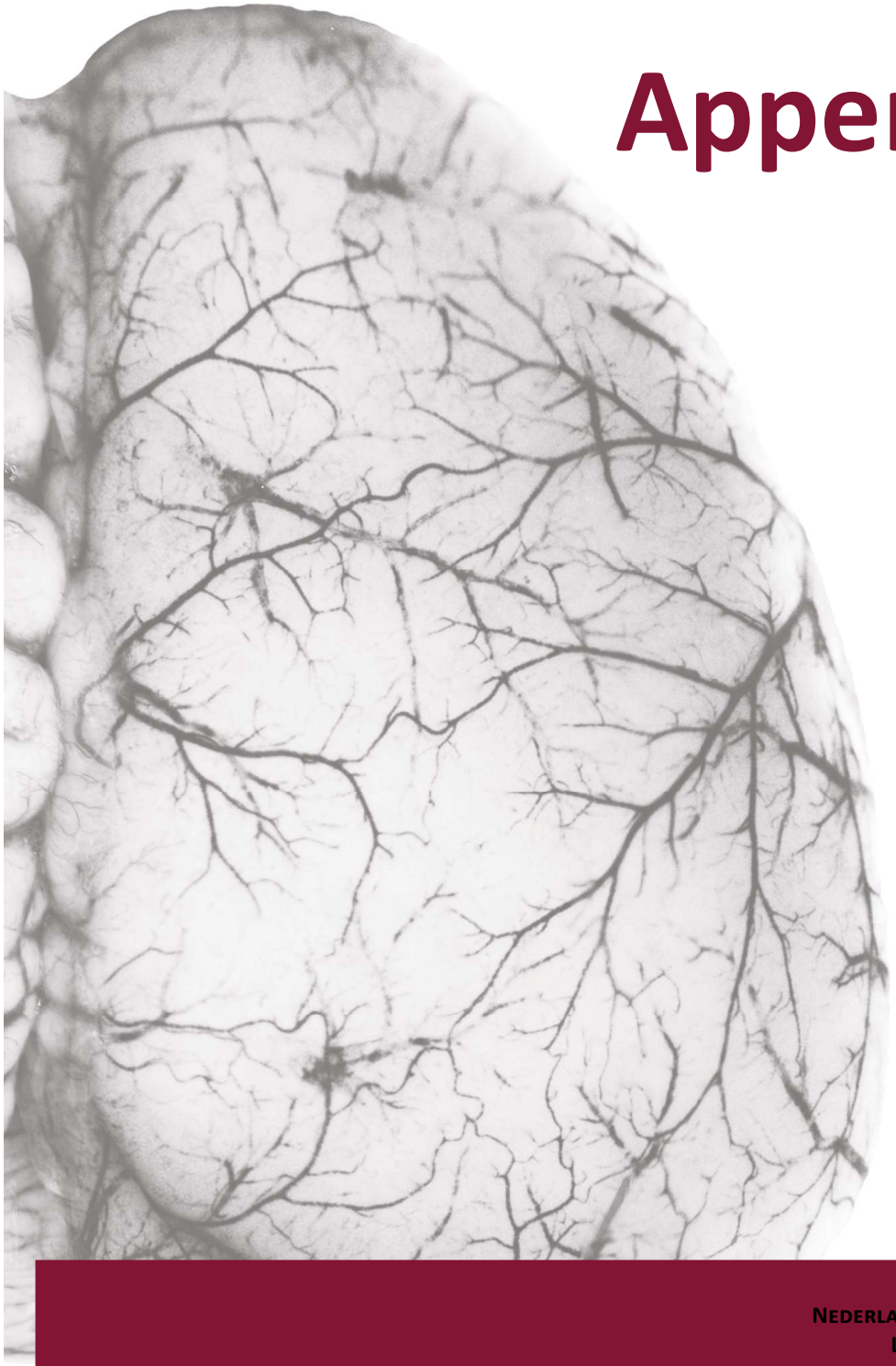

Appendix

SUMMARY

The research described in this thesis was aimed at identifying and understanding biological mechanisms and molecular pathways involved in the pathophysiology of stroke and migraine, including the detrimental connection between them. The thesis consists of two parts. Part 1 describes multiple experimental stroke research projects in mice in which we set out to: (I) improve the methodology of stroke research, and (II) unravel the stroke-migraine connection using different research strategies, methods, and transgenic mouse models. The mouse models express human pathogenic mutations found in CADASIL, RVCL-S and FHM1 and represent the clinical spectrum of monogenic disorders linking ischemic stroke and migraine. Part 2 includes multiple clinical projects in which we set out to study a large cohort of ischemic stroke patients with and without migraine in search for means to investigate stroke characteristics and vascular pathology.

Part I describes the pre-clinical stroke research. In experimental stroke research, tissue preparation is key for obtaining reliable results, as rapid post-mortem changes can negatively influence the analysis of molecular compounds in affected tissue from MSI approaches that detect changes in peptide and metabolite composition with detailed spatial resolution.
Chapter 1 provides a general introduction to the topics described in this thesis.
In Chapter 2 the methodology to sacrifice and process mouse brains for MSI analysis was optimized in a way less sensitive to *post mortem* degradation. Two methods were compared namely *ex vivo* heat stabilization and *in situ* funnel-freezing, of which *ex vivo* heat stabilization is most widely used in experimental stroke research (but prone to *post mortem* degradation). *In situ* funnel-freezing, in which liquid nitrogen is poured over the intact scull (from which the skin had been removed) of the animal has the benefit that blood circulation is maintained until the cold reaches a vessel so compounds are frozen almost instantly, so time for degradation is kept at a minimum. Direct comparison of both sacrificing and two processing methods (that involve thawing of samples so they can be analysed with MSI apparatus) revealed that *post mortem* metabolic stability in brain sections was best maintained using *in situ* funnel-freezing in combination with fast thaw-mounting. This methodology was used for experiments described in Chapter 3.
In Chapter 3 the MCAO model was used to investigate the specific molecular lipid signature after experimental transient infarction in various areas of affected (core, penumbra) and healthy brain tissue in WT and FHM1 mice that harbour the human pathogenic R192Q missense mutation. At 4, 8 and 24 hours after infarct onset, mice were sacrificed using the *in situ* funnel-freezing technique of Chapter 2 and brain sections were analysed using TOF-SIMS MSI, MALDI MSI and MS/MS. Infarct evolution was clearly shown by 2D renderings of multiple (lyso-) phosphatidylcholines isoforms. The penumbra was especially visible starting at 8 hours post-MCAO by m/z = 965.5 and m/z = 1045.5 corresponding to Phosphatidylinositolsphosphate (PIP_1) and Phosphatidylinositol 4,5-bisphosphate (PIP_2) which could be an interesting observation as they may serve as early ischemic markers and provide information on tissue faith in the penumbra. Together with the finding of increased sodiated species in FHM1 punumbra compared to increased potassiated species in the WT penumbra might reflect an increased ischemic vulnerability of penumbral tissue especially in migraine mutant mice. The observed changes may help our understanding of the mechanisms involved in the first hours after stroke onset.
In Chapter 4 a novel method was developed to calculate experimental infarct volume in

mice from T2-weighted MRI images in a manner that is less labour intensive than manual demarcation of stroke regions, which is common practice in experimental stroke research. Quantitative analysis of data from manual thresholding and tracing not only takes a lot of time but is also subjected to potential researcher bias. Hence, a fully automated approach was developed for the analysis of longitudinal MRI data to quantify ischemic lesion volume progression in the mouse brain, which reduces the analysis time by more than 10-fold and avoids researcher bias. The method is based on a level-set lesion segmentation algorithm that is built using a minimal set of assumptions and requires only one MRI sequence (T2) as input. The method shows good agreement with manual segmentation and is accurate on heterogeneous data of various time points (4, 24, 48 hours and 8 days), different MRI hardware (7T Pharmascan Bruker Biospin and 11.7T Biospec Bruker BioSpin) and acquired by centers in Leiden and Cologne. This method was thereafter used to calculate the infarct volumes described in Chapters 5 and 6.

In Chapter 5 mortality, stroke susceptibility and vascular characteristics were investigated in a recently generated transgenic knock-in mouse model with a human C-terminal frameshift mutations in the *TREX1* gene. In patients, such mutations result in RVCL-S that is characterized by vasculopathy, especially in densely vascularized organs, white matter lesions, ischemic stroke and migraine. In line with the severe phenotype in patients, homozygous mutant mice show an increased mortality starting at midlife. The mutant mice also showed a vascular phenotype as evidenced by attenuated PORH responses (across all age groups) and lower acetylcholine-induced relaxations in aortae (in 20- to 24-month-old mice). A vascular phenotype is also suggested by the increased infarct volume seen in 12- to 14-month-old mutant mice at 24 hours after infarct onset. Therefore, these RVCL-S KI mice (showing increased mortality, signs of abnormal vascular function, and increased sensitivity to experimental stroke) can be instrumental to investigate the pathology seen in RVCL-S patients.

In Chapter 6 ischemic stroke and migraine characteristics were investigated in transgenic mouse models with pathogenic gene mutations for FHM1 (neuronal mechanisms), CADASIL and RVCL-S (vascular mechanisms). Cortical spreading depolarization (CSD) and MCAO, as experimental surrogates of migraine and ischemic stroke, respectively, were induced in young (3- to 6-month-old; both CSD and MCAO) and older (12- to 14-month-old; only MCAO) FHM1, CADASIL and RVCL-S mutant mice. Only FHM1 mutant mice showed abnormal, increased CSD frequency and propagation rate. Anoxic depolarization latency was decreased in both age groups of FHM1 and CADASIL mutant mice. Infarct volume was increased only in RVCL-S mutant mice (an effect that was mainly driven by the older group of mice) and that correlated with increased neurologic deficit after infarct. Both CSD as well as vascular dysfunction and the interplay between the two seem to play an important role in monogenic diseases as FHM1, CADASIL and RVCL-S since increased CSD susceptibility does not always result in increased infarct volume and *vice versa*, increased infarct volume cannot always be attributed to increased CSD susceptibility.

Part II describes the clinical research aiming to investigate stroke- and migraine-related infarct characteristics, vascular dysfunction and spreading depression in several patient cohorts.

Patients with a history of migraine might be susceptible for spreading depolarizations which are known to affect vascular and neuronal function and penumbra recovery after stroke. In an observational study in Chapter 7 we investigated whether these patients have more brain damage in the first days after stroke and less favourable outcome after recanalization therapy compared with patients without migraine. To do so, we included patients from a prospective

multicenter ischemic stroke cohort. Lifetime migraine history was based on International Classification of Headache Disorders-II criteria. Patients underwent non-contrast CT, CT-angiography and CT-perfusion ≤ 9 hours of stroke onset and follow-up CT after 3-5 days. In total, we included 600 patients of whom 43 had migraine (24 with aura). Patients with migraine had as often as patients without migraine a perfusion deficit on admission, similar infarct core area, a penumbra area, mean blood-brain barrier permeability, malignant edema, hemorrhagic transformation, final infarct volume and poor clinical outcome after 3 months. Our results suggest that patients with migraine do not have significantly more secondary brain damage, poor outcome after ischemic stroke and have similar effect after recanalizing treatment, compared with patients without migraine.

A possible mechanism for the stroke-migraine comorbidity is vascular dysfunction. Although this is an extremely broad concept of multifactorial origin, vascular dysfunction almost always involves (primary, secondary or even tertiary) arteriosclerosis. In fact, several studies have suggested that patients with migraine more often have signs of atherosclerosis in the systemic circulation. Therefore, we aimed in Chapter 8 to investigate the association between migraine and cerebrovascular atherosclerosis in a cohort of 656 patients with acute ischemic stroke. Using state-of-the-art CT imaging technique, we assessed intra- and extra-cranial atherosclerotic changes and found that migraine patients and controls did not have more atherosclerotic changes in intracranial or extracranial (semi-) large vessels. Our results suggest that the biological mechanisms by which migraine results in ischemic stroke are not related to cerebral atherosclerosis, on a macroscopic level.

Chapter 9 provides a general discussion about the main findings described in this thesis.