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Unraveling the genetic architecture of migraine: exploring the vascular components

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ADDENDUM

Summary

This thesis explores the genetic architecture of migraine, including familial hemiplegic migraine (FHM), as well as related monogenic neurovascular disorders such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S) and Dutch-type hereditary cerebral amyloid angiopathy (D-CAA), their clinical phenotype, and their pathophysiology. The ultimate goal of the thesis is to contribute towards the understanding of migraine and small vessel disease pathophysiology and identify novel treatment targets. Finally, the thesis aims to not only contribute towards the knowledge accumulated in the respective research fields, but also strives towards achieving gender equality in research, as this is not merely a matter of ethical responsibility but also enhances the quality, validity, and impact of research findings. The research is divided in three parts. **Part I** describes studies focusing on the genetics of migraine and how this may influence treatment (**Chapters 2-5**). **Part II** describes studies on monogenic vascular syndromes that have been associated with migraine (**Chapters 6-12**). Finally, **Part III** aims at identifying challenges that physicians and researchers face in the headache field (**Chapters 13 and 14**).

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

Considering the data derived from epidemiological studies in both cohorts and families it has become clear that the genetic influence in migraine with aura is more pronounced than that of migraine without aura. In **Chapter 2**, the ground was laid for studies into monogenic migraine by writing a review on the genetic underpinning of migraine with aura. Several genetic approaches have been employed to identify genetic factors conferring migraine with aura risk. Genome-wide association studies (GWAS) tested variants across the genome in a hypothesis-free manner. Whereas GWAS for the diagnosis migraine has already identified dozens of gene variants, the search for gene variants specific for migraine without aura or migraine with aura has been disappointing. Investigating all genotyped single nucleotide polymorphisms (SNPs) together, so not focusing on individual significant hits, had already proven more successful and led to the notion that migraine with aura and migraine without aura are genetically more alike than different. Importantly, most relevant genetic discoveries related to migraine with aura came from investigating monogenetic syndromes with migraine aura as a prominent phenotype (i.e., FHM and CADASIL) that pointed to roles for neurotransmission and vasculature in migraine pathophysiology. By increasing cohort size, by testing the contribution or rare variants and by studying

epigenetic factors, we are expected to increase our understanding of the genetic architecture of migraine, including migraine with aura.

In **Chapter 3** it was investigated whether DNA methylation (DNAm) changes occur when patients with chronic migraine and medication overuse (headache) respond to treatment and whether there are already differences between responders and non-responders at baseline. To this end, a longitudinal epigenome-wide association study was conducted as part of the CHronification And Reversibility of Migraine (CHARM) clinical trial. Blood was taken from patients with chronic migraine (n = 98) at baseline and after a 12-week medication withdrawal period. Treatment responders, defined as patients with ≥ 50% reduction in monthly headache days (MHD), were compared with non-responders to identify DNAm changes associated with treatment response. Likewise, patients with ≥ 50% versus < 50% reduction in monthly migraine days (MMD) were compared. The results revealed that at the epigenome-wide significant level a longitudinal reduction in DNAm at an intronic CpG site within the *HDAC4* gene was associated with MHD response following withdrawal of acute medication. HDAC4 is highly expressed in the brain, plays a major role in synaptic plasticity, and modulates the expression and release of several neuroinflammation markers that are implicated in migraine pathophysiology. Investigating whether baseline DNAm status associated with treatment response identified lower baseline DNAm at a CpG site within *MARK3* that associated with MMD response at 12 weeks. *MARK3* encodes microtubule affinity regulating kinase 3. MARKs are serine/threonine kinases that regulate numerous cellular functions such as cell polarity, cell cycle progression, glucose metabolism and cytoskeletal dynamics.

Patients with migraine often suffer from other disorders, such as epilepsy, stroke, depression and sleep disorders, but their relationship with migraine is not well understood. Especially, a clear overview of neurological and psychiatric disorders that are comorbid with migraine with an overview of the role of genetics in this was lacking. Therefore, in **Chapter 4** a literature review was conducted on common neurological and psychiatric disorders that show comorbidity with migraine. To this end, the overlap was evaluated with respect to pathophysiological mechanisms and genetic architecture. It was found that depression and migraine show bidirectional causality and share genetic factors. Dysregulation of both hypothalamic and thalamic pathways have been suggested as a possible cause. Migraine patients have an increased risk for ischaemic stroke that likely involves spreading depolarization. Epilepsy and migraine exhibit a bidirectional relationship, where neuronal hyperexcitability serves as a crucial overlapping mechanism between the two conditions. Finally, concerning the

association between sleep disorders and migraine, hypothalamic dysfunction was suggested as the underlying mechanism.

In **Chapter 5** it was investigated how patients with hemiplegic migraine and monogenic migraine disorders (CADASIL and RVCL-S) respond to acute and preventive migraine treatment. In total n=78 patients with hemiplegic migraine (n=78), mutation carriers with CADASIL (n=114) and mutation carriers with RVCL-S (n=28) were included in the study. Migraine prevalence was 53% in CADASIL and 43% in RVCL-S mutation carriers. The most effective preventives with the least side effects for hemiplegic migraine were lamotrigine, valproate, topiramate, and acetazolamide. Valproate appeared most effective in CADASIL. Acetazolamide and propranolol showed efficacy in RVCL-S. Therefore, in hemiplegic migraine and CADASIL an alternative first-line treatment should be considered comparable with treatment that is more effective for migraine with aura. Regarding acute treatment, medications such as verapamil, nimodipine, ketamine, triptans, pulse steroids, and hypertonic saline have been utilized. If over-the-counter analgesics provide inadequate headache relief, triptans may be considered for the treatment of migraine. Patients with a history of stroke or transient ischemic attack (TIA) or a history of hemiplegic or basilar migraine are often not prescribed triptans out of fear of a stroke. In our cohort, triptans were used by 86 patients from these populations without vascular side effects.

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

Calcitonin gene-related peptide (CGRP) plays a crucial role in migraine headaches. CGRP is a neuropeptide found in the nervous system, and its level increases during a migraine attack. The development of CGRP-targeted therapies represents a significant advancement in migraine treatment and has provided new options for individuals who do not respond well to traditional migraine medications. Anti-CGRP(-receptor) antibodies are designed to prevent migraine attacks. However, as CGRP is also involved in cardiovascular regulatory mechanisms, there have been concerns whether these drugs might give cardiovascular side-effects. Therefore, in **Chapter 6**, the importance of safety was discussed when treating migraine as part of a vascular monogenic syndrome. Treating patients with small vessel diseases such as the monogenic disorder CADASIL with these drugs until relevant safety data and long-term follow up results are available was deemed unwise. Alternative preventive migraine treatments in CADASIL may be acetazolamide, sodium valproate, lamotrigine, topiramate, verapamil, or flunarizine.

In **Chapter 7**, the role of endothelial-dependent and -independent vascular reactivity in the pathophysiology of RVCL-S and CADASIL, two cerebral small vessel diseases that are considered models for stroke, vascular dementia, and migraine was evaluated. Participants with either RVCL-S or CADASIL were investigated and compared with age-, BMI-, and sex-matched controls. Endothelial-dependent vascular reactivity was evaluated by flow-mediated vasodilatation and endothelial-independent vascular reactivity (i.e., primarily vascular smooth muscle cell function) measurement that assessed dermal blood flow in response to capsaicin application. Flow-mediated vasodilatation was decreased in participants with RVCL-S compared with controls, while no such difference was found for participants with CADASIL. Contrastingly, where vascular smooth muscle cell function was reduced in participants with CADASIL compared with controls, there appeared no large difference in vascular smooth muscle function in participants with RVCL-S. This discovery suggests that endothelial dysfunction is implicated in RVCL-S, while impairment in vascular smooth muscle relaxation is more likely to play a role in CADASIL.

The retina is considered an extension of the central nervous system and shares structural and functional similarities with the brain. Therefore, by studying the retina one can obtain unique insight into neurological conditions. Optical Coherence Tomography (OCT) is a non-invasive, user- and patient-friendly method that has shown potential to detect structural retinal changes in several neurodegenerative diseases. It provides a cross-sectional visualization of the optic disc and macula in which the different macular layers can be analyzed separately. In **Chapters 8 and 9** it was investigated whether D-CAA and RVCL-S lead to changes in retinal thickness using OCT. In **Chapter 8**, carriers of an *APP* mutation carriers, a mutation that leads to D-CAA, and controls were compared. Symptomatic mutation carriers were defined as having a history of ≥1 symptomatic intracerebral hemorrhage. The overall thickness of the peripapillary retinal nerve fiber layer (pRNFL) was decreased in symptomatic but not presymptomatic D-CAA mutation carriers compared with controls. Both pre- and symptomatic carriers had a thinner temporal-superior quadrant of the pRNFL compared to controls. Total macular volume or the thickness of the individual layers of the macula did not differ between mutation carriers and controls. **Chapter 9** explores the retinal structure in RVCL-S. As patients with RVCL-S do not only suffer from neurological complaints, but also develop visual impairment due to vascular retinopathy, this is especially an interesting avenue of biomarker research. For this study, *TREX1* mutation carriers, mutation leading to RVCL-S, and controls were examined by spectral domain OCT. *TREX1* mutation carriers had a decreased

peripapillary retinal nerve fiber layer and total macular volume compared with controls. With the exception of the temporal sector, the thickness of all peripapillary sectors was decreased in *TREX1* mutation carriers. Finally, the ganglion cell layer and inner plexiform layer were also thinner in *TREX1* mutation carriers. Notably, in nine out of twelve eyes with normal fundoscopic examination, retinal thinning was already detected. So, in both D-CAA and RVCL-S there is a thinning of the peripapillary retinal nerve fiber layer already before symptom onset. Therefore, OCT findings can potentially serve as early biomarkers for D-CAA and RVCL-S and other vascular retinopathies.

Next, the vasculature of the retina was examined in RVCL-S *in vivo*. In **Chapter 10**, OCT angiography (OCT-A) was used. OCT-A is precise noninvasive imaging method that can be used to evaluate the vascular density of the superficial capillary networks in the retina and the size of the foveal avascular zone (FAZ). Our findings demonstrated that RVCL-S causes an increase in the size of the FAZ in symptomatic patients with RVCL-S. Moreover, there is a decrease in vessel density in the superficial capillary networks in the foveal region of the retina in both pre- and symptomatic patients. In the future, newly developed precise objective instruments such as OCT(-A) may, therefore, provide important tools in determining disease activity for follow-up of (common) small vessel diseases.

Next, not only the morphology of the retina and the retinal vessels, but also their function was evaluated. In **Chapter 11**, it was determined whether retinal oxygen saturation differs between patients with RVCL-S and controls. Oxygen saturation and vessel caliber was assessed in patients with RVCL-S and controls using non-invasive technology (Oxymap T1 retinal oximeter). No differences were found in arterial oxygen saturation. However, the venular oxygen saturation of patients with RVCL-S was considerably higher than in control patients. In line with these findings, the arteriovenous difference was decreased in patients with RVCL-S. Moreover, patients with RVCL-S had a reduced vessel caliber in both arterial and venular vessels. The changes in venular oxygen saturation are likely due to decreased oxygen consumption resulting from retinal atrophy. Retinal oximetry may serve as a non-invasive and innovative tool for biomarker identification, aiding in the detection and prediction of complications associated with retinal vasculopathy in RVCL-S.

In **Chapter 12,** it was investigated the neuropsychiatric status of (pre)symptomatic mutation carriers of RVCL-S and whether MRI characteristics, white matter hyperintensities (WMH) and cerebrovascular reactivity (CVR), were related to cognitive impairment, psychiatric morbidity, and functional disability in RVCL-S. *TREX1*

mutation carriers and healthy controls were asked to conduct a comprehensive set of neuropsychological and functional tests. In addition, an MRI scan was performed on each participant. Additional to T1-weighted and FLAIR sequences, CVR was determined by CO_2 inhalation and simultaneous dual-echo arterial spin labeling (ASL) MRI acquisition. Patients with RVCL-S were analyzed separated into age groups above and below 50 years of age, as this is the assumed median age of onset of notable neurological deficits. Patients with RVCL-S aged <50 years demonstrated no cognitive deterioration compared to controls. However, they already reported more depressive symptoms in absence of other signs of psychopathology. An increase in WMH load was associated with worse executive functioning and decreased cerebrovascular reactivity (CVR) in gray and white matter with overall cognitive functioning in patients older than 50. Moreover, decreased CVR in gray matter was associated with impaired memory. Therefore, increased WMH volume and decreased cerebrovascular reactivity associate with cognitive impairment in RVCL-S but cannot be considered early markers of disease. Depressive symptoms, however, do occur early in the disease course.

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

While it is extremely important that researchers take diversity, inclusion and equality into account when designing their research, one should also take these topics into account when looking at the work environment. Career barriers and harassment are known to withhold people in medicine and science from reaching their full potential. The research performed in **Chapters 13 and 14** was aimed at assessing perceived career barriers holding back professionals in the headache field (**Chapter 13**) and whether harassment a frequently occurring problem in the headache field (**Chapter 14**). To this end, a global cross-sectional online survey was performed among professionals in the field of headache. In total 580 responders completed the survey. The first part of the survey was aimed at assessing perceived career barriers in four domains: (1) professional recognition, (2) opportunities in scientific societies, (3) clinical practice, and (4) salary and compensation. Gender was to be the most important perceived barrier in almost all domains. Furthermore, the participant's country of birth surfaced as a significant obstacle preventing their engagement in international scientific societies. Finally, it was noted that career barriers varied across world regions. Our conclusion emphasizes the critical need for global recognition and action regarding persistent and ongoing gender and country-of-origin disparities among headache professionals. This recognition should extend to areas such as recruitment, retention, opportunities, mentorships, sponsorships, and career advancement. The second part of the survey focused on workplace harassment. It was demonstrated that almost half of respondents had experienced harassment;

16.1% reported sexual harassment, 40.4% verbal harassment, and 5.5% physical harassment. Importantly, women were almost seven times more likely to experience sexual harassment than men. Headache professionals, especially women, commonly experience workplace harassment throughout their careers. Our study revealed the widespread nature of this issue and underscores the urgency of implementing strategies to cultivate a healthy and safe work environment.

Finally, **Chapter 15** provides a general discussion of this thesis with suggests possibilities for future research.