Monogenic models of migraine : from clinical phenotypes to pathophysiological mechanisms

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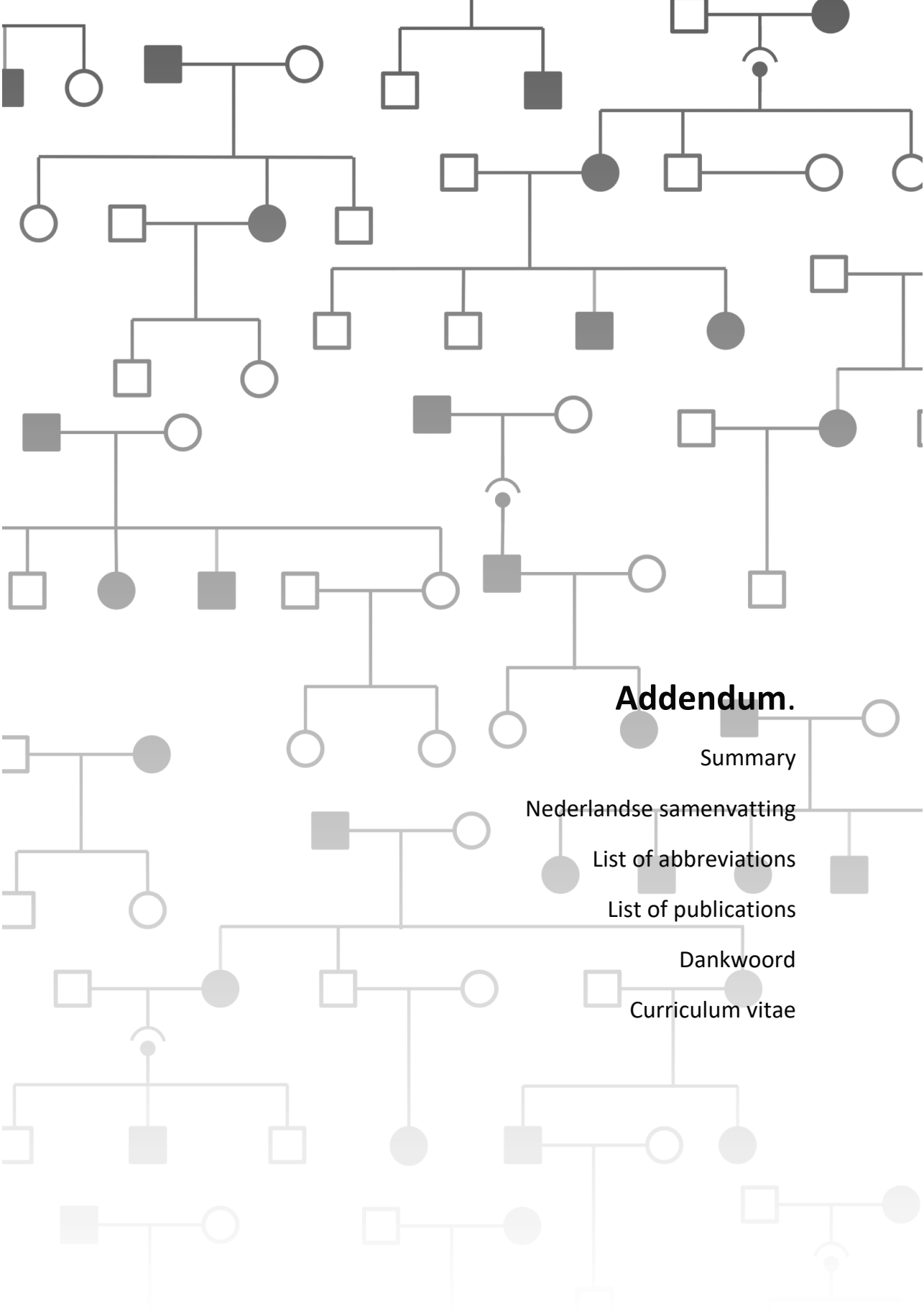

Addendum.

Summary

This thesis explores clinical phenotypes and the pathophysiology of rare monogenic models of migraine with the ultimate goal to identify novel treatment targets for these disorders, as well as for the common types of migraine. The research is divided in two parts: **part one** describes studies on hemiplegic migraine (HM), a monogenic form of migraine, **part two** describes studies on Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S), a monogenic vascular syndrome hypothesised to be associated with migraine.

Part one describes clinical genetic studies in HM. HM is a rare monogenic form of migraine with aura characterised by motor weakness during the aura phase. To date, mutations in three genes (*CACNA1A*, *ATP1A2* and *SCN1A*) are known to cause HM, either in the sporadic (SHM) or familial (FHM) form of the disease. In this thesis several phenotypic characterizations of HM patients with specific genotypes are described, which are useful to unravel the pathophysiology of specific symptoms. Furthermore, an attempt was made to identify novel HM genes.

Chapter 2 describes a review of the literature on diagnosis and treatment of HM. Various diagnostic tests are performed in HM to exclude differential diagnoses (such as stroke or epilepsy) but specific abnormalities identified by brain imaging, electroencephalography and cerebrospinal fluid analysis in the acute phase of an attack can support the diagnosis of HM. Although not tested in clinical drug trials, a review of reports on treatment of HM patients shows that flunarizine, sodium valproate, lamotrigine, verapamil, and acetazolamide may be tried as prophylactic treatment as they have shown at least some efficacy. With regard to acute treatment, fears of severe adverse effects of triptans in HM appear unfounded, and triptans should certainly be tried when common analgesics are insufficient to relieve headaches in HM.

In **chapter 3** an FHM family with a novel p.Met731Val *ATP1A2* mutation is described, in which sustained efficacy of prophylactic treatment with sodium valproate and lamotrigine was observed. The effect in this family was dramatic, certainly when considering that an attack-free state is seldom achieved with prophylactic treatment in HM. Although chances of adverse effects increase significantly when combining different prophylactics, such an approach is rather common in the field of epilepsy and can be considered in HM under strict monitoring.

Another FHM2 family with the novel p.Arg348Pro *ATP1A2* mutation is described in **chapter 4**. The 15-year prospective follow-up of this family revealed that recurrent episodes of impaired

consciousness, confusion and fever can be a prominent feature of HM. Moreover, it is discussed that the overlap of symptoms observed in this family with conditions like epilepsy, headache and neurological deficits with cerebrospinal fluid lymphocytosis (HaNDL), confusional migraine and brainstem auras indicates possible common underlying pathophysiological mechanisms for these disorders.

Chapter 5 describes the discovery of the sixth and seventh FHM-causing *SCN1A* mutations (p.Ile1498Met and p.Phe1661Leu) in two families with pure HM, that is without symptoms of cerebellar ataxia or epilepsy. This study shows that while *SCN1A* mutations are infrequently identified in HM, screening of the gene should be considered in HM patients when no mutations are found in the *CACNA1A* or the *ATP1A2* gene. The pathophysiological mechanisms behind these and other *SCN1A* mutations that cause epilepsy syndromes are complicated, but especially the p.Ile1498Met *SCN1A* mutation provides some clues that decreased function of the neuronal voltage-gated $Na_V1.1$ sodium channels may be the underlying mechanism causing FHM.

In 2012, *PRRT2* was proposed as the fourth HM gene, which is critically analysed in **chapter 6**. The HM-*PRRT2* association is questioned as most *PRRT2* mutation carriers do not suffer from HM, but from paroxysmal kinesigenic dyskinesia, benign familial infantile seizures (BFIS), or infantile convulsion choreoathetosis syndrome. More importantly, if the association is valid, it appears to be different from associations observed with *CACNA1A*, *ATP1A2* and *SCN1A* as large families showing autosomal dominant inheritance of HM with a *PRRT2* mutation are lacking. It is suggested that *PRRT2* may be a genetic cofactor that under certain circumstances contributes to the risk of HM.

Following up on the possible association of HM and *PRRT2*, **chapter 6** describes the re-investigation of a family with HM and BFIS in which both phenotypes were previously attributed to an *ATP1A2* mutation. Prompted by the discovery of *PRRT2* as a BFIS gene, we screened the gene in all family members and discovered a novel *PRRT2* mutation. We now conclude that in this family BFIS is caused by a *PRRT2* mutation and HM by the *ATP1A2* mutation, revoking the previously suggested association between *ATP1A2* and BFIS.

In **chapter 7** a clinical comparison of HM patients with and without a confirmed mutation in *CACNA1A*, *ATP1A2* or *SCN1A* is described, which suggested a phenotypic spectrum within HM as it is currently defined in the International Classification of Headache Disorders. The phenotype of HM patients without a confirmed mutation appears to be milder with less additional features and

thereby more similar to common migraine with aura. Furthermore, a higher age at onset and less familial clustering of HM suggests that this HM subgroup harbours a different genetic background, possibly involving complex genetic mechanisms.

To further assess the role of *PRRT2* in HM, all HM patients were screened for mutations in this gene. Peculiarly, we detected a *PRRT2* mutation in three additional FHM families, one of which again includes patients with BFIS, similar to the family described in **chapter 6**. The other two families already harbour a known pathogenic *CACNA1A* mutation that co-segregated with HM. Instead, incomplete co-segregation was observed for the *PRRT2* mutation with HM in these families. Again, these results do not support a role for *PRRT2* as an HM-causing gene via autosomal dominant inheritance as is the case for the three known HM genes. It cannot be ruled though that *PRRT2* mutations may increase the risk of developing HM via other mechanisms, foremost as a genetic modulator.

Finally, in **chapter 7**, we performed a whole exome sequencing study in 47 HM patients in whom no mutation in *CACNA1A*, *ATP1A2* or *SCN1A* had been detected. The aim was to identify novel HM genes. Unfortunately, although we generated high-quality sequencing data, we were unable to identify novel HM genes with mutations showing full, or near full, co-segregation in larger HM families. Also we did not see genes with mutations in multiple independent HM cases. However, the inability to detect novel HM genes may also point to a more complex genetic background, i.e. the disease being caused by multiple genetic variants each with a smaller effect size.

Part two of this thesis describes studies on Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic manifestations (RVCL-S), a monogenic vascular syndrome that has been associated with migraine and is caused by mutations in the *TREX1* gene. The aim of these studies was to characterise the clinical spectrum of RVCL-S, identify biomarkers for (progression of) the disease, and elucidate its pathophysiology. To this end, an observational cross-sectional case-control study was performed in the Dutch RVCL-S families: the RVCL-ID study.

Chapter 8 describes the main results of the RVCL-ID study. The systematic evaluation of RVCL-S patients in a wide age range allowed detailed descriptions and quantifications of previously identified symptoms of RVCL-S, but also identification of novel symptoms. While an increased risk ratio for Raynaud's phenomenon is confirmed in *TREX1* mutation carriers, the association with migraine and RVCL-S was not as clear as previously suggested. However, results suggest that RVCL-S

patients may be at risk of developing migraine at a later age, possibly indicating that migraine occurs secondary to vascular pathology in RVCL-S. From approximately age 40, systemic involvement of internal organs was found. Presence of liver and kidney dysfunction as well as anaemia were confirmed and shown to occur independently of traditional vascular risk factors (i.e. hypertension, smoking, high body mass index, diabetes mellitus), which were not more prevalent in patients compared with family members that did not carry the *TREX1* mutation. A novel finding was the presence of increased concentrations of thyroid stimulating hormone (TSH), indicating subclinical hypothyroidism in RVCL-S. This finding further solidifies the hypothesis of systemic involvement of highly vascularised organs in RVCL-S. Neurological involvement was most prominent in RVCL-S patients over 50 years of age, but neurological deficits were generally mild and limited to functions of fine motor control until the very last stage of RVCL-S. However, the high number of RVCL-S patients who had been declared incapacitated for work may suggest presence of mild cognitive impairment and/or psychiatric disturbances. Only the oldest included RVCL-S patient (aged 65) showed clear signs of dementia. Additionally, markers of inflammation were increased, pointing towards a role of systemic inflammation of the (small) vasculature in the pathophysiology of RVCL-S.

In **chapter 9** a pathophysiological study on RVCL-S is described, for which blood samples were collected in the RVCL-ID study to measure circulating markers of endothelial function. Marked increases in circulating von Willebrand Factor antigen (VWF:Ag), von Willebrand Factor propeptide (VWFpp) and angiopoietin-2 (Ang-2) were observed in RVCL-S patients, especially in those aged ≥40 years, which is the age around which the disease becomes clinically evident. These results confirm that the endothelium plays an important role in RVCL-S pathophysiology and that VWF and Ang-2 are (early) biomarkers of disease activity, which levels may predict clinical progression of RVCL-S and may even constitute future therapeutic targets.

Chapter 10 describes a study in which the role of *TREX1* mutations was investigated in cerebral vascular disorders without a 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. In a cohort of 100 patients in whom *NOTCH3* mutations were excluded, a heterozygous *TREX1* missense mutation was identified in two patients. While no RVCL-S-related *TREX1* mutations (i.e. C-terminal truncation) were identified, these results suggest a broader role for *TREX1* in early-onset cerebrovascular disease.

Finally, **chapter 11** provides a general discussion and suggests possibilities for future research.