Summary

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MIGRAINE IS A HIGHLY PREVALENT, disabling brain disorder affecting approximately 15% of the population. Cluster headache is another primary headache disorder with a lifetime prevalence of 0.12%. Despite the availability of prophylactic and acute therapies, treatment is not effective in many patients with these headache disorders. There is a clear need to develop better treatments but this is hampered by a lack of knowledge of the underlying molecular disease mechanisms. The research described in this thesis was aimed at elucidating some of the molecular genetic mechanisms of these two headache disorders by means of clinical and genetic studies in complex and/or monogenic forms of the disease and in related disorders. Hopefully, knowledge that will come from studies like these can be used to the benefit of patients by improving clinical diagnoses and/or by providing useful drug targets for future drug development strategies.

Chapters 2 and 3 and an additional genetic study from our group describe four novel gene mutations in patients with pure familial hemiplegic migraine (FHM) (**Chapter 2**), a mixed phenotype of hemiplegic migraine and alternating hemiplegia of childhood (AHC) (**Chapter 3**), and benign familial infantile convulsions (additional study). In **Chapter 2** the identification is described of p.Ile1498Met p.Phe1661Leu missense mutations in the *SCN1A* gene in two Spanish families with FHM. These mutations, only the sixth and seventh FHM-causing mutations in this gene thus far, underline the importance of *SCN1A* testing in FHM families. Mutation carriers in these two families showed pure hemiplegic migraine with large variability in attack severity and frequency, which is in agreement with earlier findings in patients with other FHM mutations, be it in this gene or the other two hemiplegic migraine genes. Although no functional characterization of the mutation has been performed, the fact that the p.Ile1498Met mutation affects the important and evolutionary well-conversed so-called IFMT motif of the $Na_V1.1$ channel, and thus likely affects the inactivation properties of the channel, seems to add to existing evidence from functional studies on other FHM-causing *SCN1A* mutations that decreased channel activity is the most likely cause of FHM3 patients. The majority of the mutations in *SCN1A* are however not associated with FHM, but instead lead to severe childhood epilepsy. How particular mutations in this gene lead to one of these two different allelic disorders remains to large extent a mystery. In **Chapter 3** the involvement of two genes previously implicated in AHC in patients with hemiplegic migraine is investigated. No mutations were identified in the *ATP1A3* gene, the gene previously was found to be the major genetic factor causing AHC, but an *SLC2A1* mutation was found in a severely affected patient with hemiplegic migraine, intellectual disability, seizures and exercise-induced dystonia. Mutations of the glucose transporter 1 that is encoded by *SLC2A1* had been shown to lead to low glucose concentrations in the brain and leading to a wide spectrum of paroxysmal and permanent neurological symptoms. Our patient expands

the phenotypic spectrum with hemiplegic migraine attacks. In the additional genetic study we report a common heterozygous truncating mutation in the *PRRT2* gene in three families with BFIC, an autosomal dominantly inherited epilepsy syndrome with onset between 3 and 12 months of age. Similar truncating mutations in the *PRRT2* gene, which codes for proline-rich transmembrane protein 2, were first identified in patients with paroxysmal kinesigenic dyskinesia with or without infantile convulsions. With this study the phenotypic spectrum of *PRRT2* mutations is extended to benign familial infantile convulsions without associated paroxysmal dyskinesia. Although the exact function of the protein is still unknown, it is speculated that PRRT2, through binding to synaptic protein SNAP25, may be involved in vesicle docking and calcium-triggered neuronal exocytosis. Mutant PRRT2 protein may result in abnormal neurotransmitter release and neuronal hyperexcitability. This functional phenotype resembles the hyperexcitability phenotype seen with FHM mutations, in particular in FHM1. One can speculate whether *PRRT2* mutations are also found in FHM, which would provide an explanation why BFIC and FHM can co-occur in patients. **Chapters 2 and 3** and the additional genetic study together show that there seems to be a spectrum of clinical phenotypes, related to (common) migraine, that are associated with mutations in the *SLC2A1*, *ATP1A3*, *SCN1A* and *PRRT2* genes.

Chapters 4 and 5 describe the validation of our recruitment strategy for patients with migraine and cluster headache that can be used, and has been used in the case of migraine, for large-scale genetic and clinical studies. In **Chapter 4** we reported on the successful recruitment of some 2,400 self-reported migraine patients for our Leiden University Medical Centre Neuro-Analysis program (LUMINA) study. Good correlation was shown of our questionnaire-based migraine diagnoses with interview diagnoses. Virtually all patients meeting the screening criteria had a migraine diagnosis, but prediction of aura status by the ICHD-2 criteria-based algorithm was less accurate. To construct a prediction rule for aura status, 33 items were selected from the LUMINA questionnaire and a stepwise forward logistic regression analysis was run. A seven-question subset provided higher sensitivity, slightly lower specificity, and similar positive predictive value in assessing aura when comparing with the ICHD-2-based algorithm. The migraine questionnaire has been successfully used already in recent genome-wide association studies (GWAS) in which LUMINA samples played an important role in the identification of migraine susceptibility gene variants.

In **Chapter 5** a similar project named the Leiden University Cluster headache Analysis (LUCA) program is presented, launched for recruitment of self-reported cluster headache patients for genetic and clinical studies. For this study, 437 self-reported cluster headache

patients meeting our screening criteria were recruited. Semi-structured telephone interviews revealed a high positive predictive value of an algorithm-based cluster headache diagnosis. Despite its high accuracy, the LUCA questionnaire is too long to be applied in large-scale population-based studies focusing on multiple disorders at the same time. Therefore, the shorter Quick Ascertainment of Cluster Headache (QATCH) questionnaire was developed by incorporating the items with highest positive predictive value and positive likelihood ratio in a stepwise forward logistic regression model. The QATCH questionnaire contains three questions and indicates that men with headache attacks of short duration and long headache-free intervals are very likely to have CH.

In **Chapter 6** the LUCA study from **Chapter 5** was used for the largest candidate-gene association study in cluster headache thus far. The association was re-evaluated of single nucleotide polymorphism (SNP) rs2653349 in the *HCRTR2* gene that changes amino acid 1246 of the hypocretin receptor type 2 from a glycine residue into an alanine residue. Two small earlier studies had indicated that *HCRTR2* may be a genetic risk factor for cluster headache, although a third study was negative. No support for *HCRTR2* as a susceptibility gene in cluster headache was found by testing the SNP in our LUCA population. Adding the LUCA study to existing data sets in a subsequent meta-analysis, which doubled in sample size, showed, however, suggestive association. A subsequent meta-analysis including our LUCA study and without the initial study, that showed a very large protective effect (odds ratio of 0.27, which is equivalent to an odds ratio of 3.7 in case of a susceptibility-increasing variant), still showed a significance association, although the odds ratio was more modest (0.80 (equivalent to an odds ratio of 1.25 for a susceptibility-increasing variant)). Although our study could not define whether *HCRTR2* should be disregarded as a promising susceptibility gene for cluster headache, one could argue that the outcome of combining small candidate gene association studies in a meta-analysis, even if a significant result is obtained, should be treated with great caution.

Chapter 7 describes a study in the genetically isolated population from the Erasmus Rucphen Family (ERF) study that consists of 3,465 living descendants of 22 couples who had at least six children baptized in the community church between 1850 and 1900 and from whom extensive clinical and genetic data is available. In this population 360 migraine patients and 1,291 subjects without severe headache were identified. The study aimed to investigate whether atherosclerosis explains the increased risk of ischemic stroke, myocardial infarction and peripheral arterial disease that is frequently reported in epidemiological and clinical studies on migraine. The degree of atherosclerosis was evaluated by

using three non-invasive measurements of atherosclerosis but no difference was found between patients and controls. It was therefore concluded that increased atherosclerosis is an unlikely explanation for the higher rates of cardiovascular disease in migraine patients.

In **Chapter 8** the study in ERF was extended by focussing on metabolic profiling in serum using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. It was investigated whether a set of compounds could be identified that was associated with a migraine diagnosis. The study showed significant association for 14 metabolites: leucine, isoleucine, methionine, proline, serine, valine, dimethylglycine, glucose, 1,5-anhydrosorbitol, lipids (CH_3) / cholesterol, lipids (CH_2), lipids (CH_2CO), creatinine and pyruvate. Notably, these metabolites were associated with an active migraine status, i.e. patients having migraine in the last year, not with migraine *per se*, and provide a good starting point for further research into metabolic changes in migraine patients. Although currently not suitable for application in clinical practice, it is a proof of concept showing that a metabolite fingerprint of migraine status may be worth looking into in the search for a migraine biomarker.