The evolving genetic and pathophysiological spectrum of migraine

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1.0

General introduction & scope of the thesis

1.1. Migraine

Migraine is an episodic neurovascular disorder that is characterized by attacks of severe, unilateral, pulsatile headache that is often accompanied by nausea, vomiting, photo- and/or phonophobia. Typical migraine attacks last a few hours to several days.[1] Migraine can start at any age, but the age at onset usually is before the age of 50 years. The peak age at onset is between 10 to 12 years of age for males and between age 14 to 16 for females.[2] Migraine is a very common disease that is more prevalent in women than in men. The one-year overall prevalence of migraine in Western countries is around 11%; with 6-8% in men and 15-18% in women.[3-6] The median attack frequency is 1.5 per month. Approximately ten percent of migraineurs have weekly attacks.[6] The World Health Organisation (WHO) rates severe migraine among the most disabling chronic disorders.[7]

1.2. Migraine with and without aura

Migraine can be subdivided in migraine without aura (MO) and migraine with aura (MA), based on the absence or presence of an aura phase preceding the headache phase (Table 1). About one-third of the migraine patients experience an aura. The aura phase generally lasts 20-60 minutes and includes mostly visual symptoms, but symptoms can also be sensory or speech related.[8]

Currently no reliable biological markers are available for the diagnosis of migraine. Therefore, the diagnosis depends on the patient's symptom description, using the Diagnostic and Classification Criteria of the International Headache Society.[1] Patients are characterised by the recurrence of their migraine attacks (Table 1).[1] To classify as an MO patient, the patient needs to have had at least five MO attacks. An MA patient has had at least two MA attacks. Current treatment options for migraine are far from optimal and effective in only about half of the patients.[9]

Table 1. *International headache criteria for migraine without and migraine with aura* [1]

Migraine without aura
A. At least five attacks fulfilling criteria B-D
B. Headache attacks lasting 4 to 72 hours (untreated or unsuccessfully treated)
C. Headache has at least two of the following characteristics:
1. Unilateral location
2. Pulsating quality
3. Moderate or severe pain intensity
4. Aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
D. During headache at least one of the following;
1. Nausea and/or vomiting
2. Photophobia and phonophobia
E. Not attributed to another disorder

Migraine with aura

A. At least two attacks fulfilling criteria B-D
B. Aura consisting of at least one of the following, but no motor weakness:
    1. Fully reversible visual symptoms including positive features (e.g., flickering lights, spots, or lines) and/or negative features (i.e., loss of vision)
    2. Fully reversible sensory symptoms including positive features (i.e., pins and needles) and/or negative features (i.e. numbness)
    3. Fully reversible dysphasic speech disturbance
D. Headache fulfilling criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes
E. Not attributed to another disorder

1.3. Migraine is a genetic disorder

Migraine has a strong genetic component. Many patients have first-degree relatives who also suffer from migraine.[10] Population-based family studies showed that the familial risk of migraine is increased.[11,12] First-degree relatives of probands with MO have an almost 2-fold increased risk to also suffer from this disorder, but had only 1.4 times the risk of MA, compared with the general population. Instead, first-degree relatives of probands with MA had a nearly 4-fold increased risk for MA, but no increased risk for MO.[11] Studies of mono- and dizygotic twin pairs are the classical method to investigate the relative importance of genetic and environmental factors. Migraine concordance rates are between 1.5 and 2 times higher in monozygotic twins than in dizygotic twins for both MO and MA[13,14], indicating that genetic factors are important in migraine susceptibility. A large population-based twin study comprising of some thirty thousand twin pairs revealed that genetic and environmental factors had an almost equally large contribution.[15] Shared environmental factors seemed to play a minor role as shown by studies comparing twins that were raised together or apart.[16,17]

1.4. Hemiplegic migraine

An often-used approach to find genes for complex genetic disorders is to study monogenic subtypes of these disorders. A monogenetic subtype of migraine with aura exists and is called familial hemiplegic migraine (FHM). FHM is characterized by transient hemiparesis during the aura phase (Table 2)[1], which may last from several minutes to several hours or even days. FHM patients have at least one additional first-degree family member that has identical hemiplegic migraine attacks.[1] FHM can be associated with additional neurological features, including cerebellar dysfunction, epilepsy and mental retardation.[18-20]

A sporadic form of hemiplegic migraine does exist and is called sporadic hemiplegic migraine (SHM). These patients do not have affected family members.[1] The estimated population prevalence for SHM is similar to that of FHM; approximately 0.01%. The clinical symptoms of SHM patients are identical to those of FHM.[21] It is unknown whether FHM and SHM share biological pathways and genetic factors.

Table 2. *International Headache Society Criteria for Familial Hemiplegic Migraine*[1]

Familial hemiplegic migraine
A. At least two attacks fulfilling criteria B and C
B. Aura consisting of fully reversible motor weakness and at least one of the following;
1. Fully reversible visual symptoms including positive features (e.g., flickering lights, spots, or lines) and/or negative features (i.e., loss of vision)
2. Fully reversible sensory symptoms including positive features (i.e. pins and needles) and/or negative features (i.e., numbness)
3. Fully reversible dysphasic speech disturbance
C. At least two of the following:
1. At least one aura symptoms develops gradually over ≥ 5 minutes, and/or different aura symptoms occur in succession over ≥ 5 minutes
2. Each symptom lasts ≥ 5 and ≤ 24 hours
3. Headache fulfilling criteria B-D for migraine without aura begins during the aura or follows aura within 60 minutes
D. At least on first-or second-degree relative has had attacks fulfilling these criteria A-E
E. Not attributed to another disorder

1.5. Hemiplegic migraine as a model for common migraine

A considerable proportion of the FHM and SHM patients also has attacks of common migraine with or without aura, not associated with hemiparesis.[22,18,23] Furthermore, the main clinical symptoms of the headache and aura phase are similar in HM and common migraine.[24] Therefore, FHM is believed to be part of the migraine spectrum and is considered a suitable model to study the pathophysiology of common migraine. Thus, genes and pathways involved in HM can be considered candidate genes and pathways for the common forms of migraine.

1.6. Hemiplegic migraine genes

So far, three genes have been identified in families with FHM. The first FHM gene identified is *CACNA1A* (FHM1) that is located on chromosome 19p13.[25] *CACNA1A* encodes the α1 subunit of neuronal $Ca_v2.1$ (P/Q-type) voltage-gated calcium channels that are widely expressed throughout the central nervous system (CNS)[26] and the neuromuscular junction.[27] FHM1 mutations are

associated with a broad spectrum of clinical features besides hemiplegic migraine.[18] Cerebellar ataxia[28-31] and epilepsy, both during severe FHM attacks[32] or independent of FHM attacks[33,34], are not uncommon. FHM1 mutations are also identified in some sporadic patients with hemiplegic migraine.[35]

Mutations in the *CACNA1A* gene can also cause episodic ataxia type-2 (EA2)[25] and spinocerebellar ataxia type-6 (SCA6).[36] EA2 is characterized by recurrent episodes of ataxia often associated with vertigo and migrainous headache and can be triggered by exercise, fatigue, and stress.[37] Whereas FHM1 is mainly caused by missense mutations, EA2 is mostly caused by nonsense, frameshift, splice site, and sometimes missense mutations.[38] SCA6 resulting in late onset ataxia is characterized by atrophy of cerebellar Purkinje cells. SCA6 is a polyglutamine disorder caused by small extensions of a CAG repeat that is located in the 3'-end of the *CACNA1A* gene.

The second FHM gene, *ATP1A2* (FHM2), is located on chromosome 1q23.[39] It encodes the α2 subunit of sodium-potassium pumps. Most of the *ATP1A2* mutations are associated with pure FHM without additional clinical symptoms.[39-42] However, over the years, a number of FHM2 mutations have been reported that are associated with FHM and cerebellar problems[43], childhood convulsions (BFIC)[19], and epilepsy.[41] Interestingly, certain *ATP1A2* mutations were shown to be associated with non-hemiplegic migraine phenotypes, such as basilar migraine[44] and even common migraine.[45] A specific *ATP1A2* mutation was identified in a family with atypical alternating hemiplegia of childhood (AHC)[46,47], a rare brain disorder that is characterized by hemiplegia, quadriplegia and other paroxysmal phenomena, including choreoathetotic movements and nystagmus. Age at onset in AHC is typically before 18 months (but later in the AHC family with the *ATP1A2* mutation) and symptom cessation often occurs after falling asleep.[48]

The most recently identified FHM gene is the *SCN1A* (FHM3) gene, which is located on chromosome 2q24[49] and encodes the α1 subunit of neuronal voltage-gated $Na_V1.1$ sodium channels. *SCN1A* is a well-known epilepsy gene with over 150 truncating and missense mutations that are associated with childhood epilepsy (i.e., severe myoclonic epilepsy of infancy (SMEI) or generalized epilepsy with febrile seizures (GEFS+)).[50,51] The fact that not all FHM families are linked to one of the three known FHM loci implies that there are additional FHM genes to be identified.

1.7. Monogenic and complex disorders in which migraine is prevalent

Over the past years, several biological pathways have been suggested to play a role in migraine pathophysiology. Most prevailing hypotheses suggest that migraine has a vascular, a neuronal or inflammatory origin. Interestingly, migraine patients have an increased risk (comorbidity) for several diseases in which these pathways also play a role.

Vascular pathway

A vascular component in the etiology of migraine has been debated for many years[52,53] and several vascular disorders show an increased prevalence of migraine. A clear example of a monogenetic vascular disorder in which migraine can be considered part of the clinical spectrum is the autosomal dominant disorder Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL).[54] CADASIL is caused by mutations in the *NOTCH3* gene, which encodes the Notch3 receptor that plays a key role in vascular smooth muscle cell function in small arteries and arterioles of the brain.[55] Up to one-third of CADASIL patients suffer from migraine with aura, where migraine often is the presenting clinical symptom.[56] Another example of a vascular monogenic disorder in which migraine is highly prevalent is Hereditary Vascular Retinopathy (HVR).[57] HVR is primarily characterized by progressive blindness due to vascular retinopathy and can be associated with a wide range of systemic and cerebral symptoms, including cerebral infarcts and white matter hyperintensities, vascular dementia, liver and kidney dysfunction, Raynaud's phenomenon, and migraine. Two additional North American families with similar symptoms were reported[58,59], which like the Dutch HVR family were linked to the same 3p21.1-p21.3 region.[60] A family-based genetic association analysis between the 3p21 locus and migraine and Raynaud's phenomenon showed that the HVR locus gives a higher susceptibility for migraine and Raynaud's phenomenon in the Dutch HVR family.[61] Furthermore, also non-genetic vasculopathies, such as ischemic stroke and ischemic heart disease can be comorbid with migraine.[62,63]

Neuronal pathway

Especially genetic studies in monogenic FHM showed evidence for involvement of neuronal hyperexitability pathways in migraine.[64,65] Two other common brain disorders in which neuronal hyperexcitability pathways seem to play a role are epilepsy and depression. The shared neuronal hyperexcitability pathway, may well explain why both epilepsy and depression are bi-directionally comorbid with migraine.[66,67] The association between migraine and epilepsy is particularly evident for FHM. Epilepsy often observed in carriers of FHM gene mutations, and as mentioned earlier, the FHM3 *SCN1A* gene is also a well-known epilepsy gene. Moreover, six percent of patients with common migraine have epilepsy[68] and patients with epilepsy have a 2.4 times increased risk to also suffer from migraine.[69] Migraineurs also have increased risk for major depression; population-based odds ratios (ORs) range from 2.0 to 5.8, with strongest associations for MA.[70] Patients with depression have a 2.8-3.4 times increased risk for migraine.[71] The bi-directional comorbidity for these disorders suggests that epilepsy and depression have, at least in part, a shared etiology with migraine. This is strengthened by the fact that anti-epileptic and antidepressant drugs are effective in migraine patients.[72]

Inflammatory pathway

During the headache phase of a migraine attack several vasoactive neuropeptides are released in the brain. It is hypothesized that these neuropeptides can cause neurogenic inflammation.[73,74] An example of an inflammatory disorder in which migraine is prevalent is systemic lupus erythematosus (SLE). SLE is a relapse-remitting autoimmune disorder that may affect multiple organs including the brain. About 40% of SLE patients have migraine, mostly migraine with aura.[75] Studying the genetics and molecular pathways of the disorders that are comorbid with migraine may provide valuable insights in molecular mechanisms involved in migraine.

1.8. Migraine mechanisms

Although it was previously thought that migraine either had a vascular or a neurogenic origin, the current view is that migraine has a neurovascular origin (for review see Goadsby 2007).[76] Headache is not merely the consequence of painful vasodilatation, but is due to the activation of the trigeminovascular system (TGVS) that consists of meningeal and superficial cortical blood vessels that are innervated by the trigeminal nerve. The TGVS projects to the trigeminal nucleus caudalis in the brainstem, which transfers abnormal pain signals to higher order central nervous system centers giving rise to the headache. It is now well accepted that the migraine aura is not due to reactive vasoconstriction, but is neurally derived and most likely caused by the human equivalent of the cortical spreading depression (CSD) of Leao.[77,78] In experimental animals, CSD is characterized by a short-lasting, intense wave of neuronal and glial cell depolarization that starts in the occipital (visual) cortex and spreads slowly to frontal regions of the cortex at a rate of approximately 2-5 mm/min and that is accompanied by massive fluxes of ions (Ca^{2+}, Na^{+}, and K^{+}) followed by a longer-lasting inhibition of spontaneous and evoked neuronal activity (for review see Somjen 2002).[79] The electrophysiological changes are associated with changes in cerebral blood flow (CBF). There is a considerable body of clinical evidence that CSD is the likely basis of the migraine aura. Visual aura symptoms in humans[80-82] typically spread from the centre of the visual field to the periphery with a propagation rate comparable to CSD evoked in experimental animals. Positive (e.g. scintillations, paraesthesia's) and negative (e.g. scotomata, paresis) phenomena of the migraine aura can be explained by the initial transient hyperexcitation front of CSD followed by neuronal depression. Most importantly, however, functional neuroimaging studies in humans using blood-oxygen level dependent (BOLD) signals have convincingly demonstrated that CBF changes that occur during a migraine aura are very similar to those observed in experimental animals during CSD.[83]

Animal studies have shown that CSD can activate the TGVS, and thus might trigger headache mechanisms.[84] However, the connection between CSD and headache in patients remains an open question.[85,86] For instance, it would not explain how the headache phase is triggered in the majority of migraine patients that never experience an aura. Also, the fact that ketamine treatment can reduce aura symptoms but fails to prevent the headache[87] would argue against a key role of CSD in triggering the headache. Although one can hypothesize that spreading depression may occur in these patients in clinically silent subcortical areas of the brain without propagating to the visual cortex[6,88], this has never been demonstrated.

1.9. Functional consequences of FHM mutations

Functional effects of FHM1 mutations on $Ca_V2.1$ Ca^{2+} channels

Electrophysiological methods have been used to study the effect of FHM1 mutations on calcium channel functioning. Multiple aspects of the calcium channel are of importance for its function: expression of the channel, localization on the cell membrane, conductance of the channel, voltage-dependence of opening, closing and reopening, and duration of the open state. For electrophysiological studies of FHM1 mutations, heterologous expression systems (without endogeneous expression of $Ca_V2.1$-α_{1A}) were transfected with recombinant $Ca_V2.1$ channel components. Calcium channel parameters were measured using whole cell or single channel electrophysiology to assess the consequence of FHM1 mutations on the cellular level (i.e., the combined effect of all $Ca_V2.1$ channels at the plasma membrane) or on the single channel, respectively (for review see Pietrobon 2007). At the single channel level, FHM1 mutations open at more negative voltages and have an enhanced channel open probability, compared to wild-type channels.[90-92] This *gain-of-function* consequence FHM1 mutations results in increased neuronal Ca^{2+} influx, which would predict increased neurotransmission. At the whole cell level, however, neurons from $Ca_V2.1$-α_{1A} knockout mice[93,94] that were transfected with either wild-type or mutant $Ca_V2.1$-α_{1A} cDNA constructs, all seem to indicate a *loss-of-function* effect of FHM1 mutations.[90-92,96] Hippocampal neurons derived from $Ca_V2.1$-α_{1A} knockout mice, that were transfected with wild-type or mutant $Ca_V2.1$-α_{1A} cDNA constructs revealed a reduced neurotransmitter release and a decreased contribution of P/Q-type channels controlling neurotransmitter release.[95,96]

Functional effects of FHM2 mutations on Na,K ATPases

The functional consequences of *ATP1A2* mutations have been investigated by using various in vitro assays. The cell survival assay is a frequently used functional test that gives an indication of disease causality of *ATP1A2* mutations. Na^+,K^+-ATPase activity is necessary for cell survival. In the cell survival assay, endogenous sodium potassium pumps are inactivated by application of the drug ouabain to HeLa cells that express either wild-type or mutant α2 Na^+,K^+- ATPase cDNAs that are made insensitive to ouabain.[97] The assay tests whether transfected wild-type

or mutant α2 Na^+,K^+- ATPase cDNAs are able to rescue cell survival. Several *ATP1A2* mutations are tested using this cell survival assay and many showed mutations that have an effect on cell survival. For a few *ATP1A2* mutations, Segall and colleagues studied additional parameters, such as catalytic turnover, extracellular K^+ affinity and Na^+,K^+ ATPase kinetics.[98,99] These studies showed that certain mutations, such as FHM2 mutation T345A, that were fully functional in the cell survival assay, could show an effect on other parameters.

Functional effects of FHM3 mutations on sodium channels

Similar to functional studies for FHM1 mutations, electrophysiological methods were used to determine the functional effect of the first FHM3 mutation. FHM3 mutation p.Gln1489Lys was cloned into the highly homologous heart-specific *SCN5A* cDNA due to apparent cloning difficulties with the brain-specific *SCN1A* cDNA. Wild-type or mutant *SCN5A* cDNA was transfected into human tsA201 cells.[49] Electrophysiological measurements (whole cell recordings) revealed a 2-4-fold accelerated recovery from fast inactivation for mutant $Na_v1.5$ sodium channels compared to wild-type[49], indicating increased firing capacity of the mutant neuron.

1.10. Common pathway and increased neurotransmission

The three FHM genes fit in a common neuronal pathway. Mutations in all three FHM genes affect the transport of ions and lead to increased levels of glutamate and K^+ ions in the synaptic cleft. This may lead to an increased susceptibility for CSD.[79] Increased susceptibility for CSD could well explain the aura phase of migraine attacks. Another gene that would perfectly fit this neuronal pathway is the *SLC1A3* gene, encoding EAAT1 the excitatory amino acid transporter type 1. EAAT1 is involved in glutamate removal from the synaptic cleft.[100,101] Mutations in EAAT1 could, like the known FHM mutations, affect the glutamate levels in the synaptic cleft.

Several additional observations point at a potentially pivotal role of enhanced brain glutamate levels in the triggering of migraine attacks. For instance, (i) glutamate receptor antagonists may have acute anti-migraine activity[102]; (ii) noxious dural stimulation, as an experimental animal model for acute migraine, increases glutamate release from trigeminal ganglion neurons[103]; (iii) plasma[104] and cerebrospinal fluid[105] levels of glutamate are increased in migraineurs with and without aura in between attacks, further rising during attacks.

1.11. Migraine mouse models

Natural mouse mutants

Different natural mouse mutants with mutations in the *Cacna1a* gene exist, such as *Tottering*, *Leaner*, *Rolling Nagoya* and *Rocker*.[106-109] Missense *Cacna1a* mutations are present in the *Tottering*, *Rolling Nagoya* and *Rocker* mutants (i.e., P601L, R1262G and T1310K, respectively).[106,110,111]

The *Leaner* mutant has a rather complex *Cacna1a* mutation leading to exon skipping and the inclusion of intronic sequences in the aberrantly spliced *Cacna1a* gene.[112] These natural mouse mutants all exhibit some degree of ataxia, and except for *Rocker*, several seizure types are also present. Furthermore, dyskinesia and dystonia are part of the phenotype of *Tottering* and *Leaner*, respectively.[113,107]

Transgenic mouse models

Cacna1a-deficient knock-out mice (α_{1A}-/-) that do not express $Ca_V2.1$ Ca^{2+} channels initially appear healthy. However, at approximately 10 days after birth, these mice develop a rapidly progressive neurological deficit with specific characteristics of ataxia and dystonia, and after 3-4 weeks after birth they die.[93,94] These knock-out mice are perhaps not very useful to study migraine for several reasons: i) whereas neurotransmission in the NMJ in heterozygous seems unaffected[114], the homozygous mice have a lethal phenotype very different from migraine, ii) the knockout mutation is a *loss-of-function* mutation, unlike FHM1 mutations that are considered *gain-of-function mutations*.[89] Therefore, it seems more appropriate to study migraine pathophysiology using knock-in migraine mouse models that carry human pathogenic FHM1 mutations.

Two transgenic knock-in (KI) mouse models harbouring either the R192Q or the S218L FHM1 mutation were generated by introducing the respective mutation into the orthologous *Cacna1a* gene by homologous recombination.[115,116] These mutations were previously identified in FHM1 patients.[25,117] Whereas the R192Q mutation causes a relatively mild form of FHM, the S218L mutation is associated with a more severe form of FHM with additional neurological features (i.e., cerebellar ataxia, epilepsy and brain edema after mild head trauma). Interestingly, S218L KI mice show a complex phenotype that is very similar to that observed in S218L patients, whereas R192Q KI mice show no overt phenotype. These migraine mice models are considered valuable models to study migraine pathophysiology.

1.12. Scope and outline of the thesis

Identification and characterization of migraine susceptibility genes and pathways that are involved in the disease mechanisms are very important for understanding the pathophysiology of migraine. It may also give new insights that can be useful for drug development and treatment of migraine. Studies in this thesis focus on genetic factors and molecular pathways involved in FHM, SHM, other monogenic diseases in which migraine is prevalent, as well as the common forms of migraine. As HM is considered a suitable model for the common forms of migraine, the identification of mutations in FHM genes and investigating their functional consequences is also relevant to increase insights in the genetic and molecular background of the common forms of migraine. At the start of the thesis, three FHM genes had been identified: *CACNA1A*, *ATP1A2* and *SCN1A*.

In **Chapter 2**, several mutations in the three known FHM genes are presented with a functional characterisation using cellular assays or electrophysiological studies in cell systems. The mutations are associated with a broad spectrum of clinical symptoms, ranging from FHM and epilepsy to atypical AHC.

In **Chapter 3**, the involvement of the FHM genes in 39 sporadic patients with HM is studied. Although SHM patients are clinical indistinguishable from FHM patients, it has not been extensively studied whether FHM genes may play a major role in this form of hemplegic migraine. The mutation scan resulted in the identification of several novel DNA variants. For all variants, functional studies were performed.

Chapter 4 describes the involvement of the excitatory amino acid transporter EAAT1 in EA2 patients that were negative for mutations in the *CACNA1A* gene. EAAT1 fits the same cortical glutamate-related pathway of the three known FHM gene products. Previously, a mutation in this gene had been identified in a patient with a particularly severe clinical phenotype that included episodic ataxia and hemiplegic attacks. A novel EAAT1 mutation is identified in a EA2 patient and associated clinical and functional characteristics are described.

Previously, a Dutch family with a vascular monogenic disorder; Hereditary Vascular Retinopathy (HVR), was together with two additional North American families with similar symptoms linked to chromosome 3p21. In **Chapter 5.1**, the *TREX1* gene is identified as the causative gene in these three families. Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL) is the novel name for this vascular disorder with autosomal dominant inheritance, that is characterized by progressive blindness due to vascular retinopathy that can be associated with a wide range

of clinical symptoms, including migraine. *TREX1* mutations are also identified in several other vascular and immune-related disorders, including Systemic Lupus Erythomatosis (SLE). **Chapter 5.2** describes the first *TREX1* mutation in a patient with *neuropsychiatric* SLE (NPSLE).

The rapid development of high-throughput genotyping during the last years made genome-wide association studies (GWAS) a feasible and attractive method to identify genetic factors for genetically complex disorders, such as migraine. Large clinic-based migraine cohorts from various European headache clinics (**Chapter 6.1**) and population-based cohorts from the Dutch Icelandic (DICE) consortium (**Chapter 6.2**) were used for these GWA studies. The most significantly associated SNP in the clinic-based migraine GWAS was tested in several independent MA and MO replication cohorts encompassing a total amount of over 3,000 migraine patients. For the population-based study first a GWAS was performed in a large genetically isolated population from the south of the Netherlands (ERF) that was followed by meta-analysis of GWAS data from five additional cohorts.

The identification of FHM genes gave the opportunity to generate transgenic mouse models. Two *Cacna1a* knock-in migraine mouse models with specific FHM1 mutations (i.e. S218L and R192Q) were generated. These knock-in mice are considered useful tools to study migraine. In **Chapter 7**, we study the RNA expression profiles of cerebellum and occipital cortex of FHM1 knock-in mice under basal (i.e. un-triggered) conditions, to investigate whether and to which extent neurobiological differences in these migraine mice were regulated at the gene expression level.

Chapter 8 provides a general discussion of the thesis, reviewing the results and discussing future possibilities for research in migraine genetics.

References

1. Headache classification subcommittee of the international headache society. The international Classification of Headache Disorders. 2nd Edition. *Cephalalgia* 2004;24:1-160.

2. Haut SR, Bigal ME, Lipton RB (2006) Chronic disorders with episodic manifestations: focus on epilepsy and migraine. *Lancet Neurol*. 5:148-157.

3. Rasmussen BK, Jensen R, Schroll M, Olesen J (1991) Epidemiology of Headache in General-Population – A prevalence Study. *J Clin Epidemiol* 44:1147-1157.

4. Launer LJ, Terwindt GM, Ferrari MD (1999) The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology* 53:537-542.

5. Lipton RB, Steward WF (1998) Migraine headaches: Epidemiology and comorbidity. *Clin Neurosci* 5:2-9.

6. Goadsby PJ, Lipton RB, Ferrari MD (2002) Migraine--current understanding and treatment. *N Engl J Med*. 346:257-270.

7. Menken M, Munsat TL, Toole JF (2000) The global burden of disease study. Implications for Neurology. *Arch Neurol* 57:418-420.

8. Russell MB, Olesen J (1996) A nosographic analysis of the migraine aura in a general population. *Brain* 119:355-361.

9. Ramadan NM, Schultz LL, Gilkey SJ (1997) Migraine prophylactic drugs: proof of efficacy, utilization and cost. *Cephalalgia* 17:73-80.

10. Russell MB, Olesen J (1993) The genetics of migraine without aura and migraine with aura. *Cephalalgia* 13:245-248.

11. Russell MB, Olesen J (1995) Increased familial risk and evidence of genetic factor in migraine. *BMJ* 311:541-544.

12. Stewart WF, Staffa J, Lipton RB, Ottman R (1997) Familial risk of migraine: a population-based study. *Ann Neurol* 41:166-172.

13. Ulrich V, Gervil M, Kyvik KO, Olesen J, Russell MB (1999) Evidence of a genetic factor in migraine with aura: a population-based Danish twin study. *Ann Neurol* 45:242–246.

14. Gervil M, Ulrich V, Kyvik KO, Olesen J, Russell MB (1999) Migraine without aura: a population based twin study. Ann *Neurol* 46:606–611.

15. Mulder EJ, Van Baal C, Gaist D, Kallela M et al (2003) Genetic and environmental influences on migraine: a twin study across six countries. *Twin Res* 6:422-431.

16. Ziegler DK, Hur YM, Bouchard TJ jr, Hassanein RS, Barter R (1998) Migraine in

twins raised together and apart. *Headache* 38:417-422.

17. Svensson DA, Larsson B, Waldenlind E, Pedersen NL (2003) Shared rearing environment in migraine: results from twins reared apart and twins reared together. *Headache* 43:235-244.

18. Ducros A, Dernier C, Joutel A, Cecillon M et al (2001) The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. *N Engl J Med* 354:17-24.

19. Vanmolkot KR, Kors EE, Hottenga JJ, Terwindt GM et al (2003) Novel mutations in the Na^+, K^+-ATPase pump gene ATP1A2 associated with familial hemiplegic migraine and benign familial infantile convulsions. *Ann Neurol* 54:360-366.

20. Vanmolkot KR, Stroink H, Koenderink JB, Kors EE et al (2006) Severe episodic neurological deficits and permanent mental retardation in a child with a novel FHM2 ATP1A2 mutation. *Ann Neurol* 59:310-314.

21. Thomsen LL, Ostergaard E, Olesen J, Russell MB (2003a) Evidence for a separate type of migraine with aura: sporadic hemiplegic migraine. *Neurology* 60:595-601.

22. Terwindt GM, Ophoff RA, Haan J, Vergouwe MN et al (1998a) Variable clinical expression of mutations in the P/Q-type calcium channel gene in familial hemiplegic migraine. Dutch Migraine Genetics Research Group. *Neurology* 50:1105-1110.

23. Thomsen LL, Ostergaard E, Romer SF, Andersen I et al (2003b) Sporadic hemiplegic migraine is an aetiologically heterogeneous disorder. *Cephalalgia* 23:921-928.

24. Thomsen LL, Eriksen MK, Roemer SF, Andersen I et al (2002) A population-based study of familial hemiplegic migraine suggests revised diagnostic criteria. *Brain* 125:1379-1391.

25. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R et al (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. *Cell* 87:543-552.

26. Westenbroek RE, Sakurai T, Elliott EM, Hell JW et al (1995) Immunochemical identification and subcellular distribution of the alpha 1A subunits of brain calcium channels. *J Neurosci* 15:6403-6418.

27. Uchitel OD, Protti DA, Sanchez V, Cherksey BD et al (1992) P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc Natl Acad Sci USA* 89:3330-3333.

28. Ducros A, Denier C, Joutel A, Vahedi K et al (1999) Recurrence of the T666M

calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. *Am J Hum* Genet 64:89-98.

29. Battistini S, Stenirri S, Piatti M, Gelfi C et al (1999) A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia. *Neurology* 53:38-43.

30. Kors EE, Haan J, Giffin NJ, Pazdera L et al (2003) Expanding the phenotypic spectrum of the CACNA1A gene T666M mutation: a description of 5 families with familial hemiplegic migraine. *Arch Neurol* 60:684-688.

31. Alonso I, Barros J, Tuna A, Seixas A et al (2004) A novel R1347Q mutation in the predicted voltage sensor segment of the P/Q-type calcium-channel alpha-subunit in a family with progressive cerebellar ataxia and hemiplegic migraine. *Clin Genet* 65:70-72.

32. Vahedi K, Denier C, Ducros A, Bousson V et al (2000) CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy. *Neurology* 55:1040-1042.

33. Kors EE, Melberg A, Vanmolkot KR, Kumlien E et al (2004) Childhood epilepsy, familial hemiplegic migraine, cerebellar ataxia, and a new CACNA1A mutation. *Neurology* 63:1136-1137.

34. Beauvais K, Cavé-Riant F, De Barace C, Tardieu M et al (2004) New CACNA1A gene mutation in a case of familial hemiplegic migraine with status epilepticus. *Eur Neurol* 52:58-61.

35. Terwindt G, Kors E, Haan J, Vermeulen F et al (2002) Mutation analysis of the CACNA1A calcium channel subunit gene in 27 patients with sporadic hemiplegic migraine. *Arch Neurol* 59:1016-1018.

36. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T et al (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. *Nat Genet*. 15:62-69.

37. Jen JC (2008) Hereditary episodic ataxias. *Ann N Y Acad Sci*.1142:250-253.

38. Jen JC, Graves TD, Hess EJ, Hanna MG et al (2007) Primary episodic ataxias: diagnosis, pathogenesis and treatment. *Brain* 130:2484-2493.

39. De Fusco M, Marconi R, Silverstri L, Atorino L et al (2003) Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat Genet* 33:192-196.

40. Riant F, De Fusco M, Aridon P, Ducros A et al (2005) ATP1A2 mutations in 11 families with familial hemiplegic migraine. *Hum Mutat* 26:281.

41. Jurkat-Rott K, Freilinger T, Dreier JP, Herzog J et al (2004) Variability of familial hemiplegic migraine with novel A1A2 Na^+/K^+-ATPase variants. *Neurology* 62:1857-1861.

42. Kaunisto MA, Harno H, Vanmolkot KR, Gargus JJ et al (2004) A novel missense ATP1A2 mutation in a Finnish family with familial hemiplegic migraine type 2. *Neurogenetics* 5:141-146.

43. Spadaro M, Ursu S, Lehmann-Horn F, Veneziano L et al (2004) A G301R Na^+/K^+ -ATPase mutation causes familial hemiplegic migraine type 2 with cerebellar signs. *Neurogenetics* 5:177-185.

44. Ambrosini A, D'Onofrio M, Grieco GS, Di Mambro A et al (2005) Familial basilar migraine associated with a new mutation in the ATP1A2 gene. *Neurology* 65:1826-1828.

45. Todt U, Dichgans M, Jurkat-Rott K, Heinze A et al (2005) Rare missense variants in ATP1A2 in families with clustering of common forms of migraine. Hum Mutat 26:315-321.

46. Bassi MT, Bresolin N, Tonelli A, Nazos K et al (2004) A novel mutation in the ATP1A2 gene causes alternating hemiplegia of childhood. *J Med Genet*. 41:621-628.

47. Swoboda KJ, Kanavakis E, Xaidara A, Johnson JE et al (2004) Alternating hemiplegia of childhood or familial hemiplegic migraine? A novel ATP1A2 mutation. *Ann Neurol*. 55:884-887.

48. Aicardi J, Bourgeois M, Goutieres F (1995) Alternating hemiplegia of childhood: clinical findings and diagnostic criteria. In: Andermann F, Aicardi J, Vigevano F (eds). Alternating Hemiplegia of Childhood. New York: Raven Press 3-18.

49. Dichgans M, Freilinger T, Eckstein G, Babini E et al (2005) Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet* 336:371-377.

50. Meisler MH, Kearney JA (2005) Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest* 115:2010-2017.

51. Mulley JC, Scheffer IE, Petrou S, Dibbens LA et al (2005) SCN1A mutations and epilepsy. *Hum Mutat* 25:535-542.

52. Wolff HG, Marcusssen RM, Kunkle EC (1948) Studies on headache; analysis of the contractile state of the cranial vascular tree in migraine. *Trans Am Neurol Assoc*. 73:14-17.

53. Goadsby PJ (2009) The vascular theory of migraine--a great story wrecked by the facts. *Brain* 132:6-7.

54. Gladstone JP, Dodick DW (2005) Migraine and cerebral white matter lesions: when to suspect cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Neurologist* 11:19-29.

55. Joutel A, Corpechot C, Ducros A, Vahedi K et al (1996) Notch3 mutations in CADASIL, a hereditary adult-onset conditioncausing stroke and dementia. *Nature* 383:707–710.

56. Dichgans M, Mayer M, Uttner I, Brüning R et al (1998) The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol* 44:731-739.

57. Terwindt GM, Haan J, Ophoff RA, Groenen SM et al (1998b) Clinical and genetic analysis of a large Dutch family with autosomal dominant vascular retinopathy, migraine and Raynaud's phenomenon. *Brain* 121:303-316.

58. Jen J, Cohen AH, Yue Q, Stout JT et al (1997) Hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS). *Neurology* 49:1322-1330.

59. Grand MG, Kaine J, Fulling K, Atkinson J et al (1988) Cerebroretinal vasculopathy. A new hereditary syndrome. *Ophthalmology* 95:649-659.

60. Ophoff RA, DeYoung J, Service SK, Joosse M et al (2001) Hereditary vascular retinopathy, cerebroretinal vasculopathy, and hereditary endotheliopathy with retinopathy, nephropathy, and stroke map to a single locus on chromosome 3p21.1-p21.3. *Am J Hum Genet* 69:447-453.

61. Hottenga JJ, Vanmolkot KR, Kors EE, Kheradmand Kia S et al (2005) The 3p21.1-p21.3 hereditary vascular retinopathy locus increases the risk for Raynaud's phenomenon and migraine. *Cephalalgia* 25:1168-1172.

62. Etminan M, Takkouche B, Isorna FC, Samii A (2005) Risk of ischaemic stroke in people with migraine: systematic review and meta-analysis of observational studies. *BMJ* 330:63.

63. Kurth T, Gaziano JM, Cook NR, Logroscino G et al (2006) Migraine and risk of cardiovascular disease in women. *JAMA* 296:283-91.

64. Moskowitz MA, Bolay H, Dalkara T (2004) Deciphering migraine mechanisms: clues from familial hemiplegic migraine genotypes. *Ann Neurol* 55:276-280.

65. Barrett CF, van den Maagdenberg AM, Frants RR, Ferrari MD (2008) Familial hemiplegic migraine. *Adv Genet* 63:57-83.

66. Breslau N, Schultz LR, Stewart WF, Lipton RB et al (2000) Headache and major depression: is the association specific to migraine? *Neurology* 25:308-313.

67. Fasmer OB (2001) The prevalence of migraine in patients with bipolar and unipolar affective disorders. *Cephalalgia* 21:894-899.

68. Andermann F, Andermann E (1992) Migraine and epilepsy, with special reference to the benign epilepsies of childhood. *Epilepsy Res Suppl* 6:207-214.

69. Ottman R, Lipton RB (1994) Comorbidity of migraine and epilepsy. *Neurology* 44:2105-2110.

70. Lipton RB, Hamelsky SW, Kolodner KB, Steiner TJ, Stewart WF (2000) Migraine, quality of life, and depression: a population-based case- control study. *Neurology* 55:629-635.

71. Breslau N, Davis GC, Schultz LR, Peterson EL. (1994) Joint 1994 Wolff Award Presentation. Migraine and major depression: a longitudinal study. *Headache* 34:387-393.

72. Sacco S, Olivieri L, Bastianello S, Carolei A (2006) Comorbid neuropathologies in migraine. *J Headache Pain* 7:222-230.

73. Moskowitz MA (1992) Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. *Trends Pharmacol Sci* 13:307–311.

74. Johnson KW, Bolay H (2006) Neurogenic inflammatory mechanisms. In: Olesen J, Goadsby PJ, Ramadan NM, Tfelt-Hansen P, Welch KMA. editors. The headaches, 3rd edn. Philadelphia: Lipincott Williams & Wilkins 309-319.

75. Glanz BI, Venkatesan A, Schur PH, Lew RA, Khosbin S (2001) Prevalence of migraine in patients with systemic lupus erythematosus. *Headache* 41:285-289.

76. Goadsby PJ (2007) Recent advances in understanding migraine mechanisms, molecules and therapeutics. *Trends Mol* Med 13:39-44.

77. Leao AA (1944) Spreading depression of activity in the cerebral cortex. *J Neurophysiol* 7:359-390.

78. Lauritzen M (1994) Pathophysiology of the migraine aura. The spreading depression theory Brain 17:199-210.

79. Somjen GG (2002) Ion regulation in the brain: implications for pathophysiology. *Neuroscientist* 8:254-267.

80. Lashley KS (1941) Patterns of cerebral integration indicated by the scotomas of migraine. *Arch Neurol Psychiatry* 46: 331–339.

81. Milner PM (1958) Note on a possible correspondence between the scotomas of migraine and spreading depression of Leão. *Electroencephalogr Clin Neurophysiol* 10:705.

82. Russell MB, Iversen HK, Olesen J (1994) Improved description of the migraine aura by a diagnostic aura diary. *Cephalalgia* 14:107-117.

83. Hadjikhani N, Sanchez Del Rio M, Wu O, Schwartz D et al (2001) Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc Natl Acad Sci USA* 98:4687-4692.

84. Bolay H, Reuter U, Dunn AK, Huang Z et al (2002) Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. *Nat Med* 8:136-142.

85. Blau JN (1992) Classical migraine: symptoms between visual aura and headache onset. *Lancet* 340:355-356.

86. Goadsby PJ (2001) Migraine, aura, and cortical spreading depression; why are we still talking about it. *Ann Neurol* 49:4-6.

87. Kaube H, Herzog J, Käufer T, Dichgans M, Diener HC (2000) Aura in some patients with familial hemiplegic migraine can be stopped by intranasal ketamine. Neurology 55:139-141.

88. Haerter K, Ayata C, Moskowitz MA (2005) Cortical spreading depression: a model for understanding migraine biology and future drug targets. *Headache Currents* 2:97-103.

89. Pietrobon D (2007) Familial hemiplegic migraine. *Neurotherapeutics* 4:274-284.

90. Hans M, Luvisetto S, Williams ME, Spagnolo M et al (1999) Functional consequences of mutations in the human alpha1A calcium channel subunit linked to familial hemiplegic migraine. *J Neurosci* 19:1610-1619.

91. Tottene A, Fellin T, Pagnutti S, Luvisetto S et al (2002) Familial hemiplegic migraine mutations increase Ca(2+) influx through single human CaV2.1 channels and decrease maximal CaV2.1 current density in neurons. *Proc Natl Acad Sci USA* 99:13284-13289.

92. Tottene A, Pivotto F, Fellin T, Cesetti T et al (2005) Specific kinetic alterations of human $Ca_V2.1$ calcium channels produced by mutation S218L causing familial hemiplegic migraine and delayed cerebral edema and coma after minor head trauma. *J Biol Chem* 280:17678-17686.

93. Jun K, Piedras-Rentería ES, Smith SM, Wheeler DB et al (1999) Ablation of P/Q-type Ca(2+) channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)-subunit. *Proc Natl Acad Sci USA* 96:15245-15250.

94. Fletcher CF, Tottene A, Lennon VA, Wilson SM et al (2001) Dystonia and cerebellar atrophy in Cacna1a null mice lacking P/Q calcium channel activity. *FASEB J* 15:1288-1290.

95. Cao YQ, Piedras-Renteria ES, Smith GB et al (2004) Presynaptic Ca^{2+} channels compete for channel type-preferring slots in altered neurotransmission arising from Ca2+ channelopathy. *Neuron* 43:387-400.

96. Barrett CF, Cao YQ, Tsien RW (2005) Gating deficiency in a familial hemiplegic migraine type 1 mutant P/Q-type calcium channel. *J Biol Chem* 280:24064-24071.

97. Koenderink JB, Zifarelli G, Qiu LY, Schwarz W et al (2005) Na,K-ATPase mutations in familial hemiplegic migraine lead to functional inactivation. *Biochim Biophys* Acta 1669:61-68.

98. Segall L, Scanzano R, Kaunisto MA, Wessman M et al (2004) Kinetic alterations due to a missense mutation in the Na,K-ATPase alpha2 subunit cause familial hemiplegic migraine type 2. *J Biol Chem* 279:43692-43696.

99. Segall L, Mezzetti A, Scanzano R, Gargus JJ et al (2005) Alterations in the alpha2 isoform of Na,K-ATPase associated with familial hemiplegic migraine type 2. *Proc Natl Acad Sci USA* 102:11106-11111.

100. Kanner BI, Schuldiner S (1987) Mechanism of transport and storage of neurotransmitters. *CRC Crit Rev Biochem* 22:1-38.

101. Attwell D, Mobbs P (1994) Neurotransmitter transporters. *Curr Opin Neurobiol* 4:353-359.

102. Andreou AP and Goadsby PJ (2009) Therapeutic potential of novel glutamate receptor antagonists in migraine Expert opinion on investigational drugs 18(6):789.

103. Goadsby PJ and Classey JD (2000) Glutamatergic transmission in the trigeminal nucleus assessed with local blood flow. *Brain Res* 875(1-2):119.

104. Ferrari MD, Odink J, Bos KD, Malessy MJ, Bruyn GW (1990) Neuroexcitatory plasma amino acids are elevated in migraine. *Neurology* 40(10),1582-1586.

105. Martínez F, Castillo J, Rodríguez JR, Leira R, Noya M. (1993) Neuroexcitatory amino acid levels in plasma and cerebrospinal fluid during migraine attacks. *Cephalalgia* 13 (2), 89-93.

106. Green MC, Sidman RL (1962) Tottering--a neuromusclar mutation in the mouse. And its linkage with oligosyndacylism. *J Hered* 53:233-237.

107. Meier H, MacPike AD (1971) Three syndromes produced by two mutant genes in the mouse. Clinical, pathological, and ultrastructural bases of tottering, leaner, and heterozygous mice. *J Hered* 62:297-302.

108. Oda S (1973) [The observation of rolling mouse Nagoya (rol), a new neurological mutant, and its maintenance (author's transl)] *Jikken Dobutsu* 22:281-288.

109. Zwigman TA, Neumann PE, Noebels JL, Herrup K.J (2001) Rocker is a new variant of the voltage-dependent calcium channel gene Cacna1a. *Neurosci* 15;21:1169-1178.

110. Fureman BE, Jinnah HA, Hess EJ (2002) Triggers of paroxysmal dyskinesia in the calcium channel mouse mutant tottering. *Pharmacol Biochem Behav* 73:631-637.

111. Mori Y, Wakamori M, Oda S, Fletcher CF et al (2000) Reduced voltage sensitivity of activation of P/Q-type Ca2+ channels is associated with the ataxic mouse mutation rolling Nagoya (tg(rol)). *J Neurosci* 20:5654-5662.

112. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr et al (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects *Cell* 87:607-617.

113. Noebels JL, Sidman RL (1979) Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. *Science* 204:1334-1336.

114. Kaja S, Van De Ven RC, Frants RR, Ferrari MD et al (2008) Reduced ACh release at neuromuscular synapses of heterozygous leaner Ca(v)2.1-mutant mice. *Synapse* 62:337-344.

115. van den Maagdenberg AM, Pietrobon D, Pizzorusso T, Kaja S et al (2004) A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. *Neuron* 41:701-710.

116. van den Maagdenberg AM*, Pizzorusso T*, Kaja S*, Terpolilli N* et al (2010) High CSD susceptibility and migraine-associated symptoms in CaV2.1 S218L mice *Ann of Neuro* 67(1):85-98.

117. Kors EE, Terwindt GM, Vermeulen FL et al (2001) Delayed cerebral edema and fatal coma after minor head trauma: role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. *Ann Neurol* 49:753-760.