CHAPTER 13

GENERAL DISCUSSION

For this thesis, clinical genetic investigations on migraine, its comorbid diseases and migraine syndromes were performed. The main migraine syndrome studied is monogenic Familial Hemiplegic Migraine (FHM) which is considered a good model for the common forms of migraine because of clinical similarities. The second migraine syndrome is Retinal Vasculopathy with Cerebral Leucodystrophy (RVCL, later termed CHARIOT), a monogenic syndrome with complex migraine as part of the clinical spectrum. Lastly, common migraine was studied by identifying migraine cases in a Dutch genetic isolate to investigate comorbidity with major depression and atherosclerosis. For depression it was assessed whether the disease may share genetic factors with migraine.

PART I: FHM: A MONOGENIC MIGRAINE SYNDROME

13.1 Genetic and clinical spectrum of FHM

Familial hemiplegic migraine (FHM) is genetically heterogeneous with mutations in the *CACNA1A* (FHM1), *ATP1A2 (*FHM2) and *SCNA1* (FHM3) genes.[1-3] Identification of *novel* and *recurrent* mutations in these genes is important to better characterize the clinical and genetic spectrum of the genes, which can have implications for genetic testing. Moreover, increasing the number of mutation carriers with a specific mutation allows for more meaningful genotype-phenotype comparisons. This thesis describes several novel FHM mutations with the corresponding clinical spectrum: i.e., *CACNA1A* missense mutation V1696F in **chapter 3**, *ATP1A2* missense mutation G855R in **chapter 5** and *SCN1A* missense mutation L263V in **chapter 6**. In addition, genotype-phenotype correlation studies were performed on *recurrent CACNA1A* missense mutations R1347Q and S218L (**chapters 2** and **4).** The results do not only have implications for FHM, but also for associated phenotypes such as ataxia, epilepsy, alternating hemiplegia of childhood and the occurrence of lethal brain edema upon mild head trauma (especially in children).

Findings in the FHM1 *CACNA1A* gene

CACNA1A encodes the α1 subunit of voltage-gated neuronal $Ca_V2.1$ (P/Q-type) calcium channels.[1] To date, almost 30 FHM1 (and/or SHM1) mutations have been identified, all involving missense mutations. A spectrum of symptoms exists ranging from pure FHM without additional clinical symptoms to FHM associated with such features like epilepsy and ataxia, and at the extreme end, trauma-triggered FHM attacks that induce cerebral edema, that can lead to death of the patients. **Chapter 2** illustrates well the broad clinical spectrum in FHM in four families carrying the FHM1 R1347Q mutation. R1347Q mutation carriers can suffer from pure FHM or from FHM with slowly progressive ataxia. Notably, attacks can be triggered by head trauma and be accompanied by altered consciousness in some. In addition, focal seizures may occur (with or without secondary generalization) during or independent of hemiplegic migraine attacks. The study also established R1347Q as the fourth recurrent FHM1 mutation, next to T666M,[1,4-8] R583Q[8-12] and S218L.[13-15] **Chapter 3** shows that screening of FHM genes is also worthwhile in patients with symptoms that are less typical for FHM. A monozygotic twin pair was shown to carry a novel *de novo* missense V1696L mutation in *CACNA1A*. The mutation causes an interesting overlap syndrome between FHM and alternating hemiplegia of childhood (AHC)[16]; an early-onset severe neurological disorder characterized by episodes of alternating hemiplegia or quadriplegia and progressive neurological features beginning before the age of 18 months. The study emphasizes that FHM and AHC may share pathophysiological mechanisms that involve calcium channel dysfunction. Previously, screening of FHM1, -2 and -3 genes[17,18] (*SCN1A* unpublished data) revealed no mutations in typical AHC patients. However, recently some 70% of typical AHC patients were shown to have a heterozygous *de novo* missense mutation in the *ATP1A3* gene[19,20] that belongs to the same family as the FHM2 *ATP1A2* gene, implicating indeed related pathophysiological mechanisms between FHM and AHC.

In **chapter 4** the severe end of the FHM disease spectrum was investigated in patients with the *CACNA1A* S218L missense mutation, which exhibit a particularly complex phenotype that is best characterized as 'early seizures and cerebral edema after trivial head trauma', which we consequently termed ESCEATHT. The combination of symptoms was recognized in two S218L patients. By reviewing 11 additional S218L mutation carriers from literature, it became clear that every mutation carrier does not exhibit all clinical features, but certainly is at risk for ESCEATHT. Consequently, clinicians need to be made aware if they see a combination of symptoms in line with ESCEATHT, even when typical FHM attacks have not occurred. Concomitantly with our study, a fourteenth patient with the S218L mutation was published confirming our hypothesis that a specific severe phenotype is indeed associated with the mutation.[13] In addition, in 2011, a fifteenth case was described linking the S218L mutation to hemiconvulsion-hemiplegia-epilepsy syndrome.[21]

Findings in the FHM2 *ATP1A2* gene

ATP1A2 encodes the α2 subunit of sodium-potassium pumps.[2] To date nearly 50 *ATP1A2* mutations have been reported, most of which are associated with pure FHM.[22,23] However, rarely, *ATP1A2* mutations are associated with for instance, cerebellar signs,[24,25] permanent mental retardation,[26] and epilepsy.[22,27] Whether these associations are coincidental or true remains to be seen. For instance, in a family in which both FHM and benign familial infantile convulsions (BFIC) segratated, an *ATP1A2* mutation was described as the causal factor for both diseases.[28] However recently it was shown that mutations in the *PRRT2* gene cause BIFC,[29] which also appeared to be the case in this family (unpublished data).

In **chapter 5** we further established the link between the *ATP1A2* gene and epilepsy by studying an FHM family in which patients carried the *ATP1A2* G855R missense mutation and had febrile seizures. The proband of this family also suffered from frequent unprovoked episodes of severe ataxia since early childhood. Whether cerebellar ataxia in FHM2 is coincidental or based on a functional effect of the underlying *ATP1A2* mutation still needs to be established.

Findings in the FHM3 *SCN1A* gene

SCN1A encodes the α1 subunit of neuronal $Na_V1.1$ voltage-gated sodium channels.[3,30] To date five FHM3 mutations have been identified. Notably, *SCN1A* is a well-known gene for childhood epilepsy, with well over 100 mutations that are associated with severe myoclonic epilepsy of infancy (SMEI) or generalized epilepsy with febrile seizures (GEFS+).[31,32] Besides, elicited repetitive daily blindness can occur in FHM3 patients with mutation Q1489H and F1499.[33,34] In contrast to the FHM1 and -2 genes, no recurrent FHM3 mutation has been reported. In **chapter 6**, for the first time, we describe a novel *SCN1A* mutation (L263V), which is associated with both FHM and epilepsy in multiple mutation carriers. This finding further expands the clinical spectrum associated with *SCN1A* mutations and strengthens molecular evidence that FHM and epilepsy share, at least in part, similar molecular pathways.

13.2 Pathophysiology of FHM gene mutations – a common pathway?

How do FHM mutations cause disease? The functional consequences of FHM1 *CACNA1A* mutations have been studied by various methodologies mainly involving electrophysiology, both in cellular models, i.e., in cell lines or cultured neurons expressing recombinant $Ca_V2.1$ channels, and in transgenic *knock-in* mouse models expressing FHM1 mutations R192Q or S218L. The R192Q mutation causes pure FHM1 without associated neurological features, whereas the S218L mutation causes severe hemiplegic migraine that can be accompanied by (sometimes fatal) brain edema (see below and **chapter 4** on ESCEATH).[35,36] The electrophysiological studies revealed that FHM1 mutations exhibit *gain-of-function* effects, namely an increased opening probability of $Ca_V2.1$ calcium channels and, thereby, an increased calcium influx through these channels[35-38] leading to enhanced neuronal activity with more cortical glutamate release.[39]

In line with the severe clinical consequences observed in S218L patients, electrophysiological studies show that particularly the S218L mutation has the largest *gain-of-function* effect (i.e., inactivation is more slow and recovery from inactivation is faster than with other FHM1 mutation).[37] Corresponding to these more extreme electrophysiological abnormalities on $Ca_V2.1$ channel function, transgenic *knock-in* mice with the S218L mutation, exhibit even a lower threshold for and a higher propagation speed of CSD waves compared with R192Q *knock-in* mice.[36,40] Notably, homozygous S218L mice exhibit a phenotype that closely resembles that in S218L patients.[15,36] Besides a transient hemiparesis after CSD events, these mice show increased mortality that is likely due to the occurrence of generalized tonic-clonic seizures, they respond more dramatic to mild head trauma, and exhibit cerebellar ataxia. The clinical similarities between patients and mice suggest that similar pathways related to an increased neuronal activity and susceptibility to CSD are the underlying cause of ESCEATHT in humans.

In contrast to FHM1, FHM2 *ATP1A2* mutations most often result in *loss-of-function* of the Na+,K^+ ATPase pump protein as was demonstrated by e.g., cell survival assays (showing partial or no cell survival in specific tests).[26] For instance, in **chapter 5** HeLa cells expressing the FHM2 G855R mutant showed a significantly reduced rate of cell survival. This indicates that the expressed mutant protein, which had been made insensitive to Na^+,K^+-ATPase activity blocker ouabain, was not able to compensate adequately for the loss of endogenous Na^+,K^+-ATPase activity, due to the action of ouabain, in the survival assay.

FHM3 *SCN1A* mutations affect the function of neuronal $Na_V1.1$ sodium channels. Electrophysiological studies of wildtype and mutant $Na_V1.1$ sodium channels showed divergent effects.[41] Two mutations, i.e., Q1489K, and L1649Q, which are associated with pure FHM in patients, showed clear *loss-of-function* effects, whereas FHM3 missense mutation L263V, which is associated with FHM and generalized tonic-clonic epilepsy in a number of mutation carriers (see **chapter 6**) exhibited *gain-of-function* features on $Na_V1.1$ channel functioning. It was hypothesized that loss of $Na_v1.1$ channel activity primarily disturbs the functioning of inhibitory neurons, where the $Na_v1.1$ channels normally are expressed[42,43] whereas gain of activity might have its predominant effect on excitatory neurons. This divergent behavior was not reported for mutations in $Na_V1.1$ channels linked to childhood epilepsy, although this has not been investigated thoroughly.

The study of the functional consequences of FHM genes has been crucial for furthering insight in the pathophysiology of FHM, and migraine in general. FHM1 mutations lead to increased neuronal calcium influx and concomitant increase of neurotransmitters, for instance glutamate in cortical neurons. Mutations in the FHM2 sodium potassium pump gene predict a reduced re-uptake of K^+ and, as a consequence, less uptake of glutamate from the synaptic cleft into glia cells. Mutations in the FHM3 sodium channel gene may also result in hyperxcitability *in vivo*, most likely due to an imbalance of excitatory and inhibitory neurotransmitter release. Thus, FHM mutations all convert to a mechanism of increased cerebral levels of K^+ and glutamate in the synaptic cleft, which would increase neuronal excitability, and thereby can explain the increased susceptibility to CSD. CSD plays an important role in initiating aura symptoms,[44] and, at least in experimental animal models, can activate the TGVS,[45] although it is unclear whether this occurs in humans.

Functional studies may increase insight in pathways that can explain the association of FHM with other diseases, such as in ESCEATHT (as already discussed), epilepsy and ataxia. Like FHM, idiopathic monogenic forms of epilepsy, often result from mutations in voltage-gated ion channels, such as those for sodium, potassium and chloride.[46] Spreading depolarizations occur in epilepsy.[47] Based on this, comorbidity of FHM and epilepsy likely can be explained by the fact that both diseases result from a disturbed balance between excitatory and inhibitory (cortical) mechanisms, which is, at least in a proportion of patients, related to ion channel dysfunction. Overlapping mechanisms may also explain the therapeutic overlap between these diseases. Indeed, for some of the currently available prophylactic migraine treatments that are originally prescribed as anti-epileptic drugs (e.g., valproate and topiramate), experimental evidence in rats has shown that chronic daily, but not acute, administration dose and duration-dependently suppressed KCl-induced CSD frequency by 40–80%, and increased the triggering threshold for inducing CSD.[48]

Why a proportion of FHM1 patients show slowly progressive ataxia is at present unclear. In FHM1 patients that show ataxia, cerebellar atrophy can be seen on MRI and in homozygous S218L mice, which have an ataxic phenotype, alterations in Purkinje cell morphology have been found. It has been suggested, that these morphological alterations may result from changes in intracellular calcium concentration and lead to a disturbed firing pattern of Purkinje cells causing ataxia.[36]

The knowledge of functional consequences could serve as a starting point in the development of new migraine therapies. For example, a drug that shifts the activation of $Ca_v2.1$ channels to more depolarized voltages may inhibit CSD and thereby prevent or abort migraine attacks. In line with the central role of increased release of glutamate in FHM patients and mouse models, a glutamate receptor antagonist may reduce severity and duration of aura symptoms and perhaps headache. Indeed, Ketamine, an N-methyl-D-aspartate receptor antagonist, was shown to reduce severity and duration of aura symptoms in FHM patients,[49] but more effective drugs with less side effects are needed.[50]

13.3 FHM as a model for common migraine

An important reason to study a rare migraine subtype such as FHM is to ultimately be able to translate findings to more common forms of migraine. Clinical similarities suggest that

shared pathophysiological mechanisms may underlie both types of migraine, although there is debate to what extent this is the case. For instance, several genetic studies investigated the role of FHM1 and -2 genes in the common forms of migraine, with inconclusive results; that is they reported some evidence for the involvement of *CACNA1A* [51-53] or *ATPA1A2*[54], while others found no such evidence.[55-57] Admittedly, many of these studies were severely underpowered. Still, a large candidate gene study investigating over 150 ion transporter genes in a large cohort of migraine patients provided no evidence for a major involvement in migraine susceptibility.[58] Recent genome-wide association studies (GWAS) reproduced these genetic data as there are many SNPs that cover the FHM1-3 gene regions that did not reveal significant association with common migraine.[59-62] It shows that genes in rare monogenic and common multifactorial forms of migraine may differ, but this does not imply that the central conclusion of a key role of for instance glutamate, does not apply to common migraine. The fact that the GWAS studies surfaced several genes (i.e., *PGCP, MTDH, LRP1*) that are involved in glutamate signaling, indicates that there may indeed be shared molecular disease pathways in common migraine and FHM.

In a Danish study, two known triggers for migraine without aura, i.e., CGRP (calcitonin gene-related peptide) and GTN (glyceryl trinitrate; an nitric oxide donor), failed to induce more migraine aura or headaches in FHM1 and -2 patients compared to controls. This result was claimed as argument against such shared pathophysiology.[63-65] However, given the fact that nitric oxide *prevents* CSD events, a lack of a clear effect of GTN in FHM patients (in which CSD is very prominent and likely the main trigger of attacks), is not surprising.[66] It merely shows that the triggering paradigm (CGRP and GTN administration) has different effects in common migraine and FHM.

A role for ion channel involvement in common migraine, namely familial migraine with aura, came from a study by Lafreniere and co-workers, who reported an inactivating truncating mutation in TRESK (a TWIK-related spinal cord potassium channel that is encoded by the *KCNK18* gene).[67] However, a successive publication of the same group showed that heterozygous inactivating TRESK mutations also occur in control subjects, casting serious doubt on their original claim of *causality* of a TRESK mutation in familial migraine with aura.[68]

PART II: CHARIOT/RVCL: A MONOGENIC MIGRAINE-ASSOCIATED SYNDROME

13.4 The clinical and genetic spectrum of CHARIOT/RVCL

Migraine can also be part of the clinical spectrum of certain monogenic diseases, which provides unique opportunities to unravel shared pathological molecular mechanisms. The clearest example is the monogenic small vessel disease CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy), where migraine with aura, but not without aura, is present in about 20-40 % of mutation carriers, often as the presenting clinical symptom.[69] A hypothesis for the increased prevalence of migraine with aura in CADASIL could be that vascular changes make the cortex more susceptible to CSD, thereby leading to migraine with aura. This hypothesis is strengthened by the fact that a transgenic *Notch3* mouse model has an increased susceptibility to CSD.[70]

A second example of a monogenic migraine-associated syndrome is cerebral hereditary angiopathy with vascular retinopathy and impaired organ function caused by *TREX1* mutations (CHARIOT). What is now called CHARIOT was originally described in three families under different disease names and abbreviations (i.e., cerebroretinal vasculopathy (CRV)[71], hereditary vascular retinopathy (HVR)[72] and hereditary endotheliopathy, retinopathy, nephropathy and stroke (HERNS).[73] The disease loci were mapped to a sinlge locus on chromosome 3p21.1-p21.3.[74] In **chapter 8** we describe the identification of the disease gene *TREX1,* that encodes the major mammalian 3′-5′ exonuclease trex1, with disease-causing mutations that result in premature truncation of the trex1 protein. At the time of the gene discovery, the disease was termed Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL), based on limited phenotypic knowledge of the disease. Moreover, previous separate clinical descriptions suggested phenotypic variability between families and *TREX1* mutations. For example, stroke en nephropathy were thought to be specific for HERNS[73] and the development of cerebral mass lesions in the end stage of the disease were not described for HVR.[72] Therefore, in **chapter 8** we described the clinical, radiological and pathological data of eleven RVCL families with five different mutations in the *TREX1* gene (in total 78 mutation carriers were investigated). Unlike previously suggested clinical variability, our study shows remarkably shared phenotypic characteristics between different RVCL mutations and between families. Based on our data, updated diagnostic criteria were composed. In addition, because we found no evidence for leukodystrophy, we renamed the disease CHARIOT. Diagnosis of CHARIOT should be considered in families with unexplained autosomal dominant progressive vascular retinopathy accompanied by contrast-enhancing white matter cerebral mass lesions. Signs and symptoms of (mild) liver and/or kidney impairment and white matter hyperintensities can be supportive. In addition migraine, Raynaud's phenomenon, anemia and hypertension can be present.

As CHARIOT can present with retinal, cerebral, and systemic symptoms a primary consult of patients may be for different medical disciplines, such as ophthalmology, rheumatology, internal medicine and neurology. In the literature several other families with diseases that resemble CHARIOT are reported, i.e., cases with a retinopathy with cerebral mass lesions and systemic involvement. Of them, only the family reported by Niedermeyer et al.[75,76] was screened for *TREX1* mutations, but no mutation was identified, suggesting that there may be genetic heterogeneity. The disease described differs from CHARIOT in the sense that it seems to have a recessive inheritance and the two patients described also had abnormalities characteristic of Fanconi's anemia. Families reported by Gutmann and Winkler[77,78] might also be linked to the *TREX1* gene, unfortunately, no DNA is available for testing this hypothesis.

13.5 RVCL/CHARIOT as a model for migraine?

In RVCL/CHARIOT, the pathophysiological process causes a vasculopathy that affects the integrity of cerebral and systemic small vessels. The mechanism by which this vasculopathy can increase the risk of migraine is unknown. Several explanations can be put forward, such as spurious association, a shared genetic factor, or an increased risk for CSD or endothelial dysfunction; some of which may partly overlap.

Spurious

We observed a migraine prevalence of 59% among *TREX1* mutation carriers (**chapter 9**). This is more than three times higher than what is usually reported as lifetime prevalence of migraine in the Western general population using the same criteria.[79] Even assuming that none of the 35 mutation carriers, for whom no reliable information on their migraine status is available, had migraine, the migraine prevalence in RVCL/CHARIOT would still be 37%, which is still twofold higher compared with the general population. In addition, except for mutation T236fs, migraine occurred in association with every *TREX1* mutation. Therefore a spurious association seems an unlikely explanation for the co-occurrence of RVCL/CHARIOT and migraine.

Shared genetic factor

The co-occurrence with migraine may be causally related to *TREX1* mutations. In this scenario, the gene may be considered to increase the susceptibility for migraine (i.e., serve as genetic modifier). A genetic, family-based, association study demonstrated that the RVCL/CHARIOT locus indeed slightly enhances the susceptibility for migraine in a large Dutch family.[80] On the other hand, in a genetic association study with 5 polymorphisms that cover the *TREX1* gene region using two Dutch samples from the general population (i.e., the Genetic Epidemiology of Migraine study n=860 and the Erasmus Rucphen Family study n=360 cases) no major role of common *TREX1* variants was found (unpublished data). In any case, other risk factors must be involved as well.

Vascular: endothelial dysfunction

There is evidence that migraine attacks are associated with endothelial dysfunction.[81-83] Migraine prevalence seems increased in persons with polymorphisms linked to endothelial function[84-90] and associated with elevated markers of endothelial activation.[91] In **chapter 10** we showed that, in contrast to CADASIL, RVCL/CHARIOT patients have a clear dysfunctional endothelial homeostasis, this may add to the increased risk of migraine. This may also provide a biological link between migraine and the increased cardiovascular risk found in migraine patients.

Neuronal

CSD is thought to be the underlying mechanism of the migraine aura. As RVCL patients mainly suffer from migraine without aura an increased CSD susceptibility causing migraine in RVCL/CHARIOT patients seems less likely.

PART III: MIGRAINE AND COMORBIDITY IN A GENETIC ISOLATE

The study of comorbidity may provide epidemiological or biological novel insights in mechanisms involved in migraine. Various conditions are reported to be comorbid with migraine. Among these are psychiatric disorders (depression, anxiety, bipolar disorder), neurological disorders (epilepsy), vascular disorders (Raynaud's phenomenon, ischemic and hemorrhagic stroke), heart disease (patent foramen ovale (PFO), coronary heart disease) and

others such as asthma and systemic lupus erythematosus (SLE) and comorbid pain disorders (for review see Le et al., 2011[92] and Scher et al., 2005[93]) Some of the reported associations show conflicting results. Conditions that have shown a consistent positive association with migraine include are stroke,[94] depression,[95] and epilepsy.[96]

13.6 Migraine and depression

In **chapter 11** of this thesis we studied the comorbidity of migraine and depression in a genetically isolated population in the southwestern region of the Netherlands. Findings of a bidirectional influence between migraine and depression suggest common neurobiological mechanisms.[95,97-98] There is some evidence of involvement of *similar neurotransmitters* in migraine and depression, such as serotonine, dopamine and glutamate.[99-101] Also, *stress-related mechanisms* have been implicated in both disorders.[102] Finally, the increased prevalence of migraine and depression in women indicates a role for *hormonal* factors in both disorders.[103]

In ERF we identified 360 migraine cases: 209 had migraine without aura (MO) and 151 had migraine with aura (MA). Odds ratios for depression in patients with migraine were 1.29 (95% confidence interval [CI] 0.98-1.70) for MO and 1.70 (95% CI 1.28-2.24) for MA. Heritability estimates were significant for all migraine (0.56), MO (0.77), and MA (0.96).

As overlapping pathophysiological pathways have been suggested for migraine and depression, this would imply that DNA variants involved in these pathways may confer an increased risk to develop both of these diseases. In our study we found some evidence for the existence of shared genetic factors between both traits. That is heritability estimates for migraine decreased after adjustment for symptoms of depression or use of antidepressant medication, in particular for MA. Comparison of the heritability scores for depression between patients with migraine and controls showed a genetic correlation between HADS-D score and MA. This knowledge can be of importance as migraine and depression might be considered an endophenotype, which could facilitate gene identification. The general idea is that an endophenotype may be more robust and uniform than the original trait (i.e., migraine) itself, and perhaps may even reduce locus heterogeneity. This strategy has proven successful in the gene identification of schizophrenia and bipolar disorder.[104,105]

The suggestion of shared genetic factors in migraine and depression that came from our study needs to be interpreted with caution. First, our heritability for migraine with aura is rather high and exceeds prior estimates. Second, confidence intervals in our analyses are very brought, leading to, at best, only marginally significant observations. This is perhaps inevitable, even in a very large sample such as ERF with some 3000 study subject, when analyzing a binary trait (with only 330 cases). Statistical uncertainty might limit the conclusion on the small reduction of heritability after correction for depression. Thus our results clearly should be confirmed in other studies.

Two other twin studies indeed confirmed our results. In parallel with our study, a study of Schur et al estimated that 20% of variability in depression and migraine is due to shared genetic factors.[106] Also a study from Ligthart et al., showed an increased genetic correlation between anxious depression and migraine (*r*G 0.30).[107]

Having statistically established that there are indeed shared genetic factors an important question is *how to identify these factors?* Several approaches can be put forward. One approach is to identify genomic regions with linkage analysis in migraine families and explore positive genomic regions in patients with migraine and depression, or the other way around. Alternatively, an association study can be performed with cases with migraine and depression versus controls, where depression may also be used as a quantitative trait. Two studies did a first attempt to actually identify shared risk variants for migraine and bipolar disease,[108,109] but no such studies were performed with depression thus far.

13.7 Migraine and cardiovascular disease

The association between migraine and cardiovascular disease, including ischemic stroke and subclinical brain lesions is well established for migraine with aura.[110,111] The mechanism for this association, however, is less clear. In **chapter 12** we investigated whether cardiovascular risk factors and atherosclerosis may be involved, but find no evidence for this. The jury is still out on whether this is indeed true as there is discrepancy with other studies.[81,112] Other explanations and mechanisms include CSD, vascular changes that predispose to both migraine and stroke (endothelial dysfunction), migraine-specific medication, or PFO. The latter may predispose to microemboli in the cranial circulation which may lead to triggering of CSD. Notably, in an experimental rat model it was shown that emboli can trigger CSD without causing actual visible infarcts on MRI.[113]

Shared genetic factors between migraine and stroke may also play a role. Indications come from monogenic migraine syndromes that predispose to stroke, such as CADASIL. Another example is MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes)[114] in which stroke-like events are associated with migrainous headache.

Further indications for a shared underlying genetic susceptibility may come from genes that have been implicated to play a role in stroke *and* migraine, including angiotensin converting enzyme (ACE)[115,116] and genes related to endothelial function[117,118] and homocysteine metabolism.[87,115, 119,120] For example the 677C>T variant in the 5',10'-methylenetetrahydrofolate reductase (MTHFR) gene has been investigated for stroke and migraine. The T allele impairs enzyme activity and carriers have modestly increased levels of homocysteine.[121] In a meta-analysis the MTHFR 677TT genotype was associated with a modestly increased risk for ischemic stroke.[122] Similarly, the T allele has been associated to migraine with aura, which was confirmed in two meta-analysis,[115,123] but negative findings have also been reported.[119] Kurth et al. studied the mediating effect of stroke on the association between migraine and MTHFR T allele in the Women's Health Study (WHS). In contrast to other studies, a protective effect of the TT genotype for migraine with aura was observed, however in patients with migraine with aura and the TT genotype an increased risk of ischemic stroke was observed. This might implicate that the MTHFR 677TT genotype is a marker for an increased risk of ischemic stroke in patients with migraine with aura.[120] Similarly, a modulatory effect of the ACE D/I polymorphism was found in the migraine-stroke association in women.[116] Also a GWAS study was published, that provides suggestive evidence for a shared genetic etiology of migraine and ischemic stroke. In women with migraine two SNPs located in the MEPE (matrix extracellular

phosphoglycoprotein) and IRX (iroquois homeobox protein 4) gene associated with ischemic stroke.[124] The function of these variants with regard to the risk of cardiovascular disease is unknown. However no independent replication was performed for the initial association signal so results should be interpreted with caution.

13.8 Future perspectives

The clinical and genetic spectrum of FHM was explored in this thesis. As detailed knowledge on this spectrum is now available, sequence analysis of known FHM genes remains mainly relevant for genetic diagnosis. As not all FHM families are linked to FHM1-3, it is still worthwhile identifying additional FHM genes as they can provide novel and valuable insights in the pathogenesis of FHM. Therefore, the collection of novel - and extension of existing - FHM families remains important. When clinical material is available, identification of genes will be relatively 'effortless' compared to the past with currently available dense SNP arrays designed for linkage studies and the *"Next Generation Sequencing"* technology which allows for high-throughput sequencing in increasingly large samples.

Transgenic mouse models can be used as tools to study the functional consequences of mutated FHM genes *in vivo*, which may refine genotype-phenotype relations and may increase our understanding of the actions of anti-migraine drugs. These studies can be complemented by investigating biochemical compounds in Cerebrospinal Fluid (CSF) and/or with MR brain spectroscopy (MRS) of FHM and migraine patients to correlate genetic findings to biochemical changes.

The identification of the *TREX1* gene for RVCL/CHARIOT opened new routes to obtain insight in the syndrome itself and in its relation with migraine. Future investigations of progression of the disease by detailed follow-up of mutation carriers will be important. How vascular changes in RVCL/CHARIOT arise is an unanswered question, but endothelial dysfunction may be involved, as was apparent from studies in **chapter 10**. Further studies of endothelial function may include assessment of markers for endothelial function in blood and CSF, and assessment of endothelial function in cerebro by functional imaging. On a molecular level a developed Trex1 antibody could be used in immunohistochemical and electron microscopy studies, which may shed more light on the exact location of (mutated) Trex1 in cells and tissues. In a recently generated RVCL/CHARIOT *Trex1* knock-in mouse model the functional consequences of mutated Trex1 can be assessed *in vivo*. Together these initiatives hopefully contribute to the development of a treatment option for this devastating disease.

In recent years, many GWAS have been performed for complex neurological disorders,[125,126] and identification of common genetic variants for migraine has also been successful.[59-62] Most identified loci (12 in total) are located in or immerdialtely outside genes with a known function in synaptic or neuronal regulation, pain pathways and some interact with each other. Further functional studies of identified variants and correlation with endophenotypes will be of great importance to translate the genetic findings to detailed pathways and potential treatment options for patients. It is unlikely that common genetic variability entirely explains the heritability of migraine and in the coming years much is expected from exome and whole genome sequencing, as these techniques could enable the identification low frequency

intermediate and rare high risk disease alleles.[127] Finally, it is expected that a large proportion of heritability of migraine may not be accounted for by the (combined) effect of identified genomic variants, but that epigenetic, post-genomic and/or regulatory events are also involved.[128] It will be a future challenge to capture these factors.[127]

The efficient web-based diagnostic process for migraine and evolving, interactive, biobank LUMINA (Leiden University MIgraine Neuro-Analysis program),[129] that combines clinical material, DNA and CSF and imaging data of migraine patients, will be of great relevance for future large-scale multidisciplinairy studies. Whether diagnostic categories defined in clinical practice have a common underlying genetic cause remains to be seen. Yet (unknown) endophenotypes may exist which are composed of a combination of disease features, as was suggested for migraine and depression in this thesis. Therefore, collecting data on comorbidity will remain a potentially very rewarding research activity.

In conclusion, the studies in this thesis improved our understanding of migraine and comorbid conditions and provide examples of how migraine syndromes and comorbidity can be used as a tool to learn more about the genetic factors involved in migraine. Together these findings contribute to a better understanding on how migraine attacks start. It remains vital to finally translate molecular genetic findings to novel treatment options for migraine patients.

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