Familial hemiplegic migraine

An experimental genetic headache model

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1. INTRODUCTION AND AIMS OF THIS THESIS

Familial hemiplegic migraine (FHM) is a rare subtype of migraine with transient hemiplegia during the aura phase [5]. FHM is dominantly inherited, and mutation screening of families with FHM has revealed a range of different mutations associated with the FHM phenotype [6]. The mutated FHM genes code for ion transport proteins that animal and cellular studies have associated with disturbed ion homeostasis, altered cellular excitability and neurotransmitter release [7]. These mechanisms might cause FHM, and potentially also other forms of migraine.

Hypersensitivity to migraine provoking substances is a fundamental trait in patients with migraine with (MA) and without aura (MO).

It could be expected that common pathophysiological mechanisms, such as hypersensitivity to migraine provoking substances, are responsible for the clinical overlap and phenotypical similari-
Migraine is a very common, chronic neurological disorder, affecting about 6% of men and 15% to 18% of women with the highest prevalence between the ages of 25 and 55 [9]. The public health burden of migraine is high because migraine attacks are associated with temporary disability and substantial impairment in activities [10] and severe migraine is ranked in the highest disability class [11]. Migraine headache is related to substantial economic loss [12] and the widespread disability produced by migraine is therefore an important target for treatment [13]. To optimize migraine treatment, it is important to understand the basic migraine mechanisms.

The basic neurobiology of migraine is considered to be a primary brain dysfunction, leading to activation and sensitisation in the trigeminovascular system [7]. The exact nature of this brain dysfunction, however, remains elusive, and a better understanding of the molecular migraine mechanisms is clearly needed. Throbbing headache is a cardinal feature of migraine. It therefore seems contradictory that the brain substance itself is largely not pain producing [14]. Within the skull, only few structures are able to generate nociceptive signals. These include the meninges, nerves and large cerebral and pial vessels [15]. The major pain pathway from the vessels and dura mater is first (ophthalmic) division of the trigeminal nerve [16]. Vasodilatation of cerebral vessels has been considered important in the development of migraine pain [17], and vasodilator mechanisms and neuropeptides become therefore a focus in headache research [18] giving rise to the pathophysiological concept of vascular headaches [14]. Nerve fibers from the trigeminal ganglion containing vasoactive neuropeptides, such as calcitonin gene-related peptide (CGRP), substance P and others [19], surround the intracranial vessels and innervate the dura mater, forming the trigeminovascular system [20].

With the advancing studies on various vasodilators [21-24] and functional brain imaging [25, 26], it has become clear that vascular changes are not the primary cause for head pain in migraine. Migraine is therefore currently considered a neurological disorder with temporary dysfunction of the cerebral cortex [27], caused by a primary brain dysfunction, leading to activation and sensitisation in the trigeminovascular system [7] and the release of vasoactive neuropeptides such as CGRP [28, 29].

Figure 1

The trigeminovascular system. Within the skull, pain sensitivity is largely located to the meningeal blood vessels innervated by sensory afferents from the ophthalmic division of the trigeminal nerve. During migraine attacks these afferents are activated, and input from the meningeal vessels, passes through the trigeminal ganglion and synapses on second order neurons in the trigeminocervical complex, adapted from [30].

2. MIGRAINE PATHOPHYSIOLOGY

Migraine is a very common, chronic neurological disorder, affecting about 6% of men and 15% to 18% of women with the highest prevalence between the ages of 25 and 55 [9]. The public health burden of migraine is high because migraine attacks are associated with temporary disability and substantial impairment in activities [10] and severe migraine is ranked in the highest disability class [11]. Migraine headache is related to substantial economic loss [12] and the widespread disability produced by migraine is therefore an important target for treatment [13]. To optimize migraine treatment, it is important to understand the basic migraine mechanisms.

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FHM type 1 (FHM-1) is associated with missense mutations in the CACNA1A gene on chromosome 19p13, encoding the α1A subunit of calcium channels [40], and at least eighteen different missense mutations have been identified [6].

This gene encodes the pore-forming α1A subunit of the Cav2.1 (P/Q-type) voltage-gated neuronal calcium channel [40], which modulate release of neurotransmitters at peripheral [41] and central synapses [42, 43].

Familial hemiplegic migraine of type 2:
FHM type 2 (FHM-2) is associated with mutations in the ATP1A2 gene encoding the α2 subunit of a Na+, K+ ATPase [44, 45], and more than 20 mutations have been identified [6].

Familial hemiplegic migraine of type 3:
FHM type 3 (FHM-3) is associated with mutations in the SCN1A gene on chromosome 2q24 [46, 47]. The SCN1A gene encodes the α1 subunit of the neuronal voltage-gated sodium (Nav1.1) channels.

FHM as a genetic migraine model
Given the common nature of the mutated FHM genes as ion transport proteins, FHM and potentially also other forms of migraine might be caused by impairment of ion transport. FHM has many clinical similarities to migraine with (MA) and without aura (MO) [8], and many FHM-patients have MA and/or MO [8, 50]. However, it has been reported that MA and MO are not associated with any of the known FHM mutations [51-54]. Nevertheless, it could be expected that common pathophysiological mechanisms are responsible for these clinical similarities between FHM, MA and MO indicating shared neurobiological pathways.

The identification of gene mutations and better understanding of gene function and its impact on disease phenotype could potentially lead to the development of more targeted and better migraine therapies. Genetic migraine models in both animals and humans are therefore important for identifying migraine triggers and triggering mechanisms. Genotyped FHM patients are therefore unique in migraine research, because they offer us the chance to study the interplay between genotype and phenotype and may be regarded as a valuable genetic migraine model [55]. The study of the functional consequences of FHM mutations can thus be considered a logical step in a bottom-up approach to the disease. Furthermore, FHM studies might shed a light on the importance of the FHM genotype on the response to migraine provoking substances, and hopefully a better understanding of the molecular migraine pathology in both FHM and the common migraine types [56].

Animal and cellular studies:
The functional consequence of FHM mutations in the CACNA1A, ATP1A2 and SCN1A genes have been examined in cellular and animal models, and have been found to result in a change of function of the ion channels [7].

FHM-1: Calcium channels of the P/Q-type are found throughout the brain and central nervous system (CNS) [57], with a high concentration of the α1A subunit in the cerebellum [58]. Immunochemical studies point toward a subcellular location.
predominantly on the presynaptic nerve terminals, thereby suggesting an important role in neurotransmitter release at many central synapses [42]. Other calcium channels are also found within the CNS and Ca2+ channels of both P/Q-, N-, and R-type control glutamate release. However, the Ca2+ influx through the P/Q-type channels trigger neurotransmitter release more effectively than Ca2+ influx through N- or R-type channels, possibly because the P/Q-type channels are located closer to the gluta- mate release sites [59]. FHM mutations have been found to affect both the biophysical properties on the single-channel level, and the density of functional channels in the membrane [60].

In human neurons, FHM-1 mutations increase single-channel Ca2+ influx, whereas in human cerebellar granule cells a decrease in the density of functional calcium channels is found in the membrane [61].

The net phenotype of the FHM-1 mutations have thus been suggested to be one of gain-of-function for most mutations [60] because the combination of a lower activation threshold and the increase in single-channel opening probability may lead to an increased Ca2+ influx into the nerve terminals. This event may enhance the release of the excitatory transmitter glutamate and thereby lower the threshold for cortical spreading depression (CSD) in FHM-1 patients [62].

FHM-2: The ATP1A2 sodium potassium pump transports sodium ions out of the cell while importing potassium ions. This sodium export provides the steep sodium gradient essential for the transport of glutamate and calcium and the Na+,K+-ATPase thus modulates the re-uptake of potassium and glutamate from the synaptic cleft into glial cells.

All known FHM-2 mutations produce substitutions of conserved amino acids in important functional regions of the catalytic α2 subunit of the Na+,K+-ATPase [6]. The molecular effects of the FHM-2 mutations range from reduction of Na+,K+-ATPase activity [44, 63-67], abnormally functioning channels [68], to expression of fully functional but kinetically changed channels [69, 70]. Although the molecular effect of the mutations thus leads to a wide spectrum of functional changes, all mutations are associated with a common FHM-2 phenotype. A common denominator for all the tested FHM-2 mutations is the slowed or reduced activity of the α2 Na+,K+-ATPase, which has been termed functional haploinsufficiency [44].

FHM-3: The mutated gene product in FHM-3, the Nav1.1 channel, is expressed in cortical neurons [71], pointing to an important role in the generation and propagation of action potentials in the brain. This channel plays an important role in the generation and propagation of action potentials, and mutations in this gene have been associated with epilepsy [72, 73].

The molecular effects of FHM-3 mutations have been studied using the highly homologous SCN5A channel, showing faster recovery from fast inactivation at negative voltages in mutants, which could facilitate initiation and propagation of cortical spreading depression [46].

The mutated Nav1.1 channel has been associated with a pronounced but self-limiting neuronal hyper excitability but in some cases also to hypo excitability [74]. This behaviour has not been reported for the epileptogenic Na+ channel mutations, and could be typical of the migraine mutations. Others have reported that some FHM-3 mutations linked to typical FHM result in a predominantly loss-of-function phenotype, while other mutations that are also associated with epilepsy exhibit gain-of-function features [75].

A unified model of FHM mutations in migraine – the role of glutamate

A possible causal relationship might exist between cortical spreading depression (CSD) and migraine headache. Cerebral blood flow (CBF) studies during hemiplegic aura shows a spreading cortical hypoperfusion [76], similar to migraine with aura [77], which suggest that CSD is the most likely mechanism of hemiplegic aura [78, 79]. Experiments in rats show that CSD in both the cortex [80] and hippocampus [81] may activate the nociceptive trigeminal afferents, cause vasodilatation and possibly headache via a central trigeminal parasympathetic reflex thereby linking migraine aura and the triggering of migraine headache [82]. If FHM mutations lead to an increased propensity to CSD, this link between mutation and migraine phenotype would be strengthened.

In FHM-1 patients, the CACNA1A gain-of-function phenotype may increases release of glutamate and an increased susceptibility to CSD [62], providing a possible link between mutation and CSD. In-vivo studies in CACNA1A-knock-in-mouse showed decreased threshold for CSD and increased velocity of the CSD compared to the wild-type [83], giving weight to the hypothesis that genetic predisposition, CSD and migraine might be linked [62]. Based on this it seems likely that FHM-1 patients could be more susceptible to aura and headache.

The functional haploinsufficiency [44] of FHM-2 mutations may reduce the electrochemical Na+ gradient required to drive the astrocytic glutamate transporters [84], which is co-localized with the astro-glia1 α2 isotype Na+,K+-ATPase [85]. This would reduce the removal of glutamate and lower the threshold for cortical spreading depression [62]. One might therefore expect that FHM-2 patients would show a reduced threshold for CSD and thus, induction of aura. The FHM-3 mutations in the SCN1A gene may cause an increased recovery from fast inactivation. Neurons may depolarize more easily facilitating CSD, as a consequence of the rise of extra cellular glutamate and K+ levels in the brain [86].

Do mutated FHM genes cause FHM?

The FHM mutations are clearly associated with the FHM phenotype, because they co-segregate with the affected phenotype in many families [48]. More mutations have been identified in several families [87] and have not been identified in large control groups.

The finding that the mutations affects highly conserved, important, functional regions in the channel proteins [6] have
prompted the notion that the FHM-mutations are directly disease causing. A number of FHM mutations have been examined in both animal and cellular studies, and have been found to alter the function of the mutated channels. This may lead to disturbed ion homeostasis and altered cellular excitability and neurotransmitter release [6, 7, 34]. These mechanisms might cause FHM, and potentially also other forms of migraine. To compensate for potential species differences, the FHM mutations should be studied in humans to confirm how mutations affect the disease phenotype.

5. EXPERIMENTAL HEADACHE MODELS

In vitro studies have contributed in the characterization of receptors in cranial blood vessels and the identification of new possible antimigraine agents [88]. Animal models enable the study of vascular responses, neurogenic inflammation and peptide release, and thus provided leads in the search for migraine mechanisms. To overcome possible species differences all results need to be confirmed in humans. To this end, a human in vivo model of experimental headache and migraine in humans, has been developed [89].

Mechanism-based headache research has led to the identification of two important molecular pathways in migraine pathophysiology: the nitric oxide - cyclic GMP [90] and the CGRP - cyclic AMP pathways [91]. Migraine patients are hypersensitive to activation of these pathways, and antagonism of these two pathways constitutes effective migraine treatments [92, 93].

Nitric oxide and cGMP

![Figure 6](image)

Glyceryl trinitrate (GTN), which may be regarded as a prodrug for nitric oxide, induces a mild to moderate headache in healthy subjects. Migraine patients are more sensitive to nitric oxide than non-migrainous subjects, and nitric oxide plays an important role in migraine pain [94, 95]. Several substances capable of inducing experimental vascular headache do so via a common mediator which is NO or molecules in the cascade of intracellular reactions triggered by NO [96]. A good example is sildenafil (Viagra™), a selective inhibitor of cGMP-hydrolysing phosphodiesterase 5 (PDE5). Sildenafil acts exclusively by increasing cGMP and induce migraine via a cGMP-dependent mechanism, without concomitant dilatation of the middle cerebral artery [97].

CGRP and cAMP

Calcitonine-gene related peptide (CGRP) is a 37-amino acid neuropeptide [98] that activates adenylyl cyclase, thereby increasing cyclic adenosine monophosphate (cAMP) levels [99, 100]. CGRP is involved in migraine pathogenesis [101], and might be released from the cranial circulation during migraine attacks [28, 102]. CGRP activates adenylyl cyclase thereby increasing cAMP levels [99, 100]. The role of cAMP in the headache pathogenesis has been studied using cilostazol, an inhibitor of cAMP degradation and.

This study showed that increased levels of cAMP may play a role in headache and migraine pathogenesis [103]. The migraine inducing effect of CGRP can thus be attributed to hypersensitive to activation of the CGRP - cyclic AMP pathways [91].

6. IN VIVO FUNCTIONAL STUDIES OF FHM MUTATIONS IN PATIENTS

Materials and methods

In the group of FHM patients where a mutated protein is found, it is tempting to look at genotype–phenotype correlation in order to understand the functional consequences of the mutations for the phenotype. Such studies, however, are difficult because of the clinical variation and the small number of mutation carriers. If, however, a sufficiently large number of patients could be found, the genotype–phenotype studies could be undertaken.

For the studies in this thesis patients were recruited from the cohort identified in a Danish population based study, comprising 147 subjects with FHM from 44 different families [104]. The Danish cohort consists exclusively of patients with FHM-1 and FHM-2 mutations, and a large group without any known mutations. In fact, only 14% (6/42) of FHM families in the Danish FHM population showed mutations in the CACNA1A or ATP1A2 genes. All genetic analyses were done in collaboration with deCode genetics on Iceland [48]. In total 33 subjects from the Danish cohort has a known mutation, and were thus eligible for participation in these studies.

Study I: The Danish cohort consisted of 20 FHM-1 patients with a known mutation in the CACNA1A gene according to the criteria of the International headache Society [5]. We recruited 8 FHM-1 patients with R583Q and C1369Y mutations (2 M / 6 F, mean age 40 (range 27–57 years)) and 9 healthy controls (HV) (5 M / 4 F, mean age 33 (range 24–49 years)). All 20 patients were contacted and asked to participate in the study. Ten out of 20 patients declined participation for unspecified reasons, and two of the remaining 10 patients were not eligible for participation because of known cerebrovascular or cardiovascular disease. Thus, we were able to recruit 8 out of 20 patients (40 %) from the
Danish population-based cohort. The most frequent CACNA1A mutation (T666M) [105-107], was not present in any of the participating patients, but R583Q, the second most frequent mutation [108, 109] and the most prevalent Danish FHM-1 mutation [48] was represented.

Study II: The Danish cohort consisted of 13 FHM-2 patients with known mutations [48]. We recruited 8 FHM-2 patients with R202Q, R763C, V138A and L764P mutations (5 M / 3 F, mean age 45 (range 19–59 years)), and 9 HV (5 M / 4 F, mean age 33 (range 24–49 years)). Seven patients were recruited from the Danish FHM-cohort, and one patient from the Department of Neurology, Misericordia Hospital, Grosseto and University of Milan, Italy. The Danish cohort consisted of 13 FHM-2 patients with known mutations. All patients were contacted and asked to participate in the study. Six out of 13 declined participation due to unspecified reasons, and we were thus able to recruit 7 out of 13 patients (55 %) from the Danish population-based cohort. Out of the four mutations in this study, three (R202Q, R763C, V138A) were reported for the first time in the Danish population based cohort [48], and have not yet been functionally characterized. Participants for study III and IV were recruited among the participants from the first two studies.

For study III, we recruited nine FHM patients (seven FHM-1 patients with the R583Q (5) and C1369Y (2) mutations (2 M / 5 F, mean age 39 years (range 29-56 years)), two FHM-2 patients with the R202Q and R763C mutations (0 M / 2 F, mean age 38 years (range 20-56 years)) (Table 1), and ten HV (6 M / 4 F, mean age 32 years (range 23-42 years)). We were thus able to recruit 9 out of 33 patients (27 %) with known mutations from the Danish population based cohort.

For study IV we recruited 9 FHM patients (5 FHM1, 4 FHM2, mutations R583Q, C1369Y and R763C, R202Q) and 7 HV.

Experimental design
We applied similar study design for the first three studies; non-randomized, open label, active control, parallel group. The lab technicians performing the measurements were blinded in respect to patients and controls. We opted for this design to avoid the risk of losing patients to follow up in case of cross over design. All subjects received a continuous intravenous infusion of 0.5 μg/kg/min glyceryl trinitrate (GTN) over 20 min (study I and II) or 1.5 μg/min CGRP over 20 min (study III). The subjects were informed that the infusion might induce headache in some individuals, but the timing or the type of headache was not discussed. All subjects reported to the laboratory headache-free. All procedures were performed in a quiet room at a temperature of 25°C. The subjects were placed in the supine position, and a venous catheter (Venflon®) was inserted into an antecubital vein.

Cerebral haemodynamics
Middle cerebral artery blood flow velocity: The largest terminal branch of the internal carotid artery is the middle cerebral artery (MCA), which carries the blood supply to most of the lateral surface of the cerebral hemisphere. From its origin, the MCA extends laterally and horizontally in the lateral cerebral fissure.
This fixed point was then used throughout the study in each subject, and every measurement was done after carefully optimizing the signal from this point. The middle cerebral artery was chosen for measurement because of better reproducibility than measurements in the posterior or anterior cerebral arteries, as shown in previous methodological studies [113] and because the timeframe for the measurements only allowed measurements in one set of arteries during the study. Skilled technicians did all recordings.

We have previously shown that it is possible to record diameter changes in the intracerebral vessels with magnetic resonance angiography [114], but we opted for the present set-up, because it allowed recordings from both the intracranial and extracranial arteries.

Diameter of the superficial temporal artery:
Diameter of the frontal branch of the superficial temporal artery (STA) was measured by a high-resolution ultrasonography unit (Dermascan C, Cortex Technology, Denmark: 20 MHz, bandwidth 15 MHz) [115]. When the sound beam of the probe is directed perpendicular to the skin, superficially located arteries such as the temporal artery can be located. High amplitudes of the signal reflect the interfaces between blood and vessel wall. A confirmation of this can be made by gently compressing, whereby the pulsation of the vessel is seen. This helps distinguish the artery from the veins. The mean of four measurements randomly distributed within the shortest possible interval (within 1 minute) was used to ensure reliable data.

Vital signs:
Heart rate and blood pressure were measured every 10 min using an auto-inflatable cuff (ProPac Encore® Welch Allyn Protocol, Beaverton, USA). Electrocardiogram was monitored on an LCD screen (Cardiofax V, Nihon-Cohden, Japan) and recorded on paper every 10 min.

Data analysis and statistical methods

All values are presented as mean ± SD, unless otherwise stated. We defined an immediate phase as the period from 0 to 120 min after the start of infusion (0-120 min) and a delayed phase as the period from 2 h to 14 h after the start of infusion (2 h-14 h).

Baseline was defined as 10 min before the start of infusion of each dose (-10 min). The area under the curve (AUC) was used as summary measure for analyzing differences between the groups and was calculated according to the trapezium rule [116]. The primary endpoints were differences in incidence of migraine and migraine-like headache and in the AUC for headache score (AUCheadache 0-120 min and AUCheadache 2 h-14 h), Vmean-MCA (AUCVmeanMCA), STA (AUCSTA) between groups.

Calculation of sample size was based on the detection of a difference between the proportion of patients and controls reporting GTN and CGRP induced migraine or migraine-like headache attack during the delayed phase, at 5% significance with 80% power. For study I and II, we assumed that GTN would induce a migraine or migraine-like headache attack in approximately 80% of FHM-1 patients as reported previously in common types of migraine [95, 117] and migraine like headache in less than 10% of healthy controls [118, 119]. We estimated that 8 subjects should be included in each group [120].

For study III, we assumed that CGRP would induce a migraine or migraine-like headache attack in at least 50% of FHM patients as reported previously in common types of migraine [101] and migraine like headache in less than 10% of healthy controls [121]. We estimated that 9 subjects should be included in each group [120].
Electrophysiological characterization of FHM-patients

In the common forms of migraine, migraine with (MA) and without aura (MO), the brain and brain stem are characterized interictally by habituation deficits in the form of amplitude decrease of evoked responses or reflexes during repeated stimulation. This has been reported for visual, auditory, somatosensory and nociceptive evoked cortical potentials [122], and for the nociception-specific blink reflex (nBR) [123, 124]. The nociceptive blink reflex (nBR) is mediated by brainstem neurons [125], and lack of habituation of the this reflex is a reproducible abnormality found in migraineurs between attacks in evoked potential studies [126] and also in healthy volunteers with a family history of migraine [124]. Deficient nBR habituation might thus be a trait-marker for the genetic predisposition to typical migraine. If abnormal neuronal activity is associated with the FHM genotype, electrophysiological recordings could visualize this. We therefore studied the habituation of cortical and subcortical evoked responses in FHM patients [4].

7. RESULTS

Nitric oxide and FHM-1 (Paper I)

Nitric oxide is an effective and reproducible trigger of headache [127] and migraine in migraine patients [117]. We therefore used the GTN migraine provocation model to explore the repeated stimulation. This has been reported for visual, auditory, somatosensory and nociceptive evoked cortical potentials [122], and for the nociception-specific blink reflex (nBR) [123, 124]. The nociceptive blink reflex (nBR) is mediated by brainstem neurons [125], and lack of habituation of the this reflex is a reproducible abnormality found in migraineurs between attacks in evoked potential studies [126] and also in healthy volunteers with a family history of migraine [124]. Deficient nBR habituation might thus be a trait-marker for the genetic predisposition to typical migraine. If abnormal neuronal activity is associated with the FHM genotype, electrophysiological recordings could visualize this. We therefore studied the habituation of cortical and subcortical evoked responses in FHM patients [4].

GTN failed to induce a migraine aura

Cortical spreading depression (CSD), discovered by Leão [129], has been linked to migraine aura pathogenesis in both observational [130], animal [35] and human studies [36, 131, 132]. Cerebral blood flow (CBF) studies during hemiplegic aura showed a spreading cortical hypo perfusion [76], similar to migraine with aura [77], which suggest that CSD is the most likely mechanism of hemiplegic aura [78, 79]. It still remains unresolved why migraine patients are more susceptible to CSD. In the case of FHM, animal studies of CACNA1A knock-in mice showed increased susceptibility to CSD [83]. It has been proposed that CACNA1A mutations lead to increased release of glutamate, and an increased susceptibility to CSD [62].

The molecular mechanisms for the initiation and propagation of CSD are not fully understood. However, animal studies reported that CSD is associated with the release of nitric oxide [133, 134], and nitric oxide has also been linked to the modulation of the calcium entry through P/Q type calcium channels [135], and the transduction between neuronal activity and increased CBF after CSD [136]. Furthermore, Read and colleagues showed that GTN stimulates the release of nitric oxide in response to CSD [134] and CSD increases the levels of cGMP in the cortex and brain stem [137]. These findings suggest that the NO-cGMP pathway could be importantly involved in the pathogenesis of migraine aura. The i.v. GTN model was used by Christiansen et al. [119] in a study attempting to trigger migraine aura in 12 patients with pure migraine with aura, i.e. without any co-existing migraine without aura. The study showed that 50 % of the patients developed migraine headache but none of the patients developed migraine aura. In another study of 21 patients with migraine with aura, intravenously GTN induced reproducible aura in one patient [117], and in a study of 22 patients, sublingually applied GTN induced aura in 3 patients [138]. Collectively these data suggest that GTN may be able to induce aura in some migraine patients, although with a relatively low rate of aura induction. The genotype of FHM-1 may be associated with a decreased CSD threshold and it could therefore be expected that GTN might be able to induce aura in some of the FHM-1 patients. GTN failed, however, to induce migraine aura in this population-based cohort of Danish FHM-1 patients with the R583Q and C1369Y mutations [48]. Thus, based on our data, it appears that activation of the NO-cGMP pathway does not trigger aura in FHM-1 patients.

GTN failed to induce migraine headache

Experimental studies in migraineurs have demonstrated that the NO-cGMP pathway plays an important role in triggering headache [94, 95, 97, 117, 119, 139] and maintaining [92] migraine headache. Interestingly, the study by Christiansen et al. [119] showed that although GTN failed to induce aura, most MA patients developed migraine headache. This indicates that the NO-cGMP neurobiological pathway is involved in triggering migraine headache in patients with MA. FHM-1 and MA patients share clinical features such as non-hemiplegic aura symptoms, a similar headache phase and similar associated symptoms [8]. We therefore hypothesized that GTN-infusion would induce a migraine headache in most FHM-1 patients. The present study showed, however, that GTN failed to trigger more migraine headache in FHM-1 patients than in healthy controls, and the reported pain intensity was not significantly different between the groups. This is in sharp contrast to earlier findings where GTN caused more episodes of migraine and migraine-like headache in migraine patients than in controls [117, 128].
GTN have also been reported to induce a more pronounced headache response in migraine patients than in healthy controls [94, 119].

In line with previous studies on migraine patients [94], the FHM-1 patients developed more immediate headache (0-2 h) than controls. Arterial dilatation may cause headache [140], and GTN infusion causes a more pronounced dilatation of extra- and intracerebral arteries in migraine patients than in controls [141]. We found changes in the diameter of the STA, and a decrease in the VmeanMCA comparable to earlier studies using the same dose of GTN [142], but we did not detect any differences in VmeanMCA, or the diameter of the STA between FHM-1 and controls. This could indicate that FHM-1 may not share the arterial hypersensitivity to NO that has been suggested for MO patients [141]. It also shows that the difference in immediate headache between FHM-1 and controls is unlikely to be caused by vasodilatation.

Surprisingly few controls developed headache, compared to our earlier studies using the NO-cGMP model. The incidence of im-

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<thead>
<tr>
<th>Delayed headache (2-14h)</th>
<th>5</th>
<th>2</th>
<th>0.15</th>
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<tr>
<td>Migraine according to ICHD</td>
<td>1</td>
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Groups compared with Fisher’s exact test.
mediate headache in the control group, however, is similar to a large study by Sances et al. [138]. Studies on the R593Q-mutation also examined in the present study [143] showed that FHM mutated human CaV2.1 channels display an increased open probability, thus allowing FHM-1 channels to carry larger Ca2+ fluxes than in the wild type [61]. Animal studies on knock-out rats for the CaV2.1 calcium channel, indicate that the P/Q-type calcium channels may have a pronociceptive role in inflammatory and neuro-pathic pain states [144]. Based on these data, it would be plausible to suggest that the more pronounced immediate headache in the FHM-patients may be due to the pronociceptive effect of the gain-of-function phenotype known from the R539Q-mutation. These results suggest that FHM-1 patients do not show hypersensitivity of the NO-cGMP pathway, as characteristically seen in MO and MA. Furthermore, pathophysiological pathways underlying migraine headache in FHM-1 may be different from the common types of migraine (MA and MO). Our material does not allow a separate evaluation of each mutation. Further studies are warranted to examine this, and explore whether FHM-2 also differ from the common types of migraine.

Nitric oxide and FHM-2 (Paper II)
Clinically, the migraine features of FHM-1 and FHM-2 are not different, but the mutated genes may lead to different functional consequences. It is, therefore, possible that the sensitivity to migraine trigger GTN may be different between patients with FHM-1 and FHM-2. We therefore used the GTN migraine provocation model to explore the functional consequences of the R202Q, R763C, V138A and L764P gene mutations in FHM-2 patients. A common denominator for all tested FHM-2 mutations is a slowed or reduced activity of the α2 Na+,K+-ATPase, which has been termed functional haploinsufficiency [44]. Reduced activity of the α2 Na+,K+-ATPase may reduce the removal of glutamate and lower the threshold for cortical spreading depression (CSD) [62]. Pharmacological inactivation of the Na+,K+-ATPase (similar to the effects of FHM-2 mutations) causes CSD-like depolarisation [145] and stimulation of the NO - cGMP pathway has been shown to inhibit Na+,K+-ATPase activity [146, 147]. FHM-2 patients might therefore show a reduced threshold for CSD and migraine.

Migraine aura
Activation of the NO - cGMP pathway failed to induce more migraine aura in FHM-2 patients than in healthy volunteers, which is similar to patients with migraine with typical aura where GTN rarely [117] or never [119] induces aura. One patient reported hemiplegic aura and migraine headache. This patient reported that she was going through a difficult period of her life and we could be triggered again, we could have repeated the GTN-infusion as previously described in migraine with typical aura [117], but decided against it because the strain of inducing a hemiplegic attack is considerable.

Migraine headache
It is well established that MA and MO patients share a common hypersensitivity to activation of the NO - cyclic GMP pathway [94, 95, 117, 138]. We would therefore expect a robust headache response after GTN in FHM-2, because spontaneous migraine headache in FHM is similar to MA and MO [5, 8].

Surprisingly, we found no differences in the prevalence of migraine attacks fulfilling the IHS criteria between FHM-2 and controls (table 2). GTN infusion induced migraine in only 25 % of FHM-2 patients, which is much lower than what is seen in both MO and MA.

Table 2: Number of patients and controls reporting headache and migraine headache

<table>
<thead>
<tr>
<th></th>
<th>FHM-2</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate headache (0-120 min)</td>
<td>4</td>
<td>3</td>
<td>0.65</td>
</tr>
<tr>
<td>Delayed headache (2-h-14h)</td>
<td>5</td>
<td>2</td>
<td>0.15</td>
</tr>
<tr>
<td>Migraine according to ICHD</td>
<td>2</td>
<td>0</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Groups compared with Fisher’s exact test.

This finding is in sharp contrast to previous findings in patients with common types of migraine. We found no differences in area under the headache curve and vascular variables between FHM-2 and controls. The FHM-2 group did, however, report a biphasic headache response after GTN infusion (figure 14). Moreover, the median peak headache intensity was higher in patients than in controls during the immediate and the delayed phases. Although the delayed response has occasionally been reported in healthy volunteers after GTN [148], we can not rule out that FHM-2 patients may be more sensitive to the GTN provocation than healthy volunteers.

Six patients had migraine co morbidity of MO (two) and MA (four). This co-occurrence could be a determinant for NO-hypersensitivity, but to study this would require two groups of patients; one with known mutations and co-existing MA or MO versus “pure” FHM-2 patients. Such a study would be highly relevant, but difficult to set up due to the rarity of these patients.
In summary, FHM-2 patients the R202Q, R763C, V138A and L764P gene mutations do not develop migraine attacks after GTN, but we can exclude a small hypersensitivity to NO in FHM-2 patients with co-occurring MO and MA, but this effect is probably too small to be clinical relevant.

Implications
If FHM-2 patients are less sensitive to nitric oxide, it has implications for our understanding of the headache mechanisms and raises the question whether FHM-2 is distinct from the common types of migraine. The present study therefore raises the question whether the ATP1A2 mutations are causative in the migraine pathogenesis. Pathophysiological pathways underlying migraine headache in FHM-2 patients may thus be different from the pathways in patients with the common types of migraine. There might, however, be other pathways of importance for the FHM phenotype, such as the well-known migraine triggering CGRP-cAMP pathway, known from MO-patients [101], which has never been examined in FHM patients.

CGRP and FHM (Paper III)
Calcitonin gene-related peptide (CGRP) is a neuropeptide [98] that is present in structures relevant to migraine and its release dilates cephalic arteries [28, 29, 149-151]. Strong evidence of the involvement of CGRP in migraine was provided in a study where infusion of CGRP caused migraine or migraine-like headache in migraine sufferers [101] and a mild headache in healthy controls [121]. Even more importantly, CGRP-receptor antagonism has documented efficacy in the treatment of migraine attacks [152]. An orally available CGRP antagonist have been tested in randomized clinical trials and have been found both safe and efficient [93, 153].

The mechanisms underlying the migraine inducing effects of CGRP are still not known in detail, but the phenotype might be linked to the FHM mutations. Clarifying the functional consequences of FHM mutations by examining the sensitivity to known migraine provoking substances such as CGRP in a human experimental headache model, might improve our understanding of the FHM phenotype.

We therefore hypothesised that CACNA1A and ATP1A2 mutations in a group of genotyped FHM patients would be associated with hypersensitivity to CGRP, similar to that observed in patients with MO [101]. This would indicate shared migraine mechanisms in FHM and MO and a potentially important role for CGRP-antagonists in the management of FHM-patients. Surprisingly, the main outcome of the study was that CGRP infusion failed to induce more auras or more migraine-like headache in FHM patients than in healthy controls.

CGRP did not induce migraine aura
Migraine aura is likely to be the symptom of CSD [78], and a model has been proposed that links FHM mutations with a propensity to CSD [62]. Animal studies have established a link between FHM-1 mutations and increased susceptibility to CSD [83], but why migraine patients are more susceptible to CSD remains unresolved.

The present study examines the relationship between the FHM genotype, which may be associated with a reduced threshold for CSD and CGRP. The ability of CGRP to induce CSD is unknown, but this is a likely possibility since infusion of the neuropeptide endothelin-1 is able to cause CSD [154], probably via stimulation of phospholipase C [155]. CGRP acts in part via the same mechanism [156]. We found that CGRP is not able to trigger migraine aura in FHM patients and CGRP is probably not critically important in the aura pathogenesis in FHM.

CGRP is not an effective trigger of migraine headache in FHM patients
CGRP is important for pain signals in neurogenic inflammation [157] which has been linked to migraine pathogenesis [158]. Sensitivity to CGRP may be a determinant of the nociceptive threshold because nociceptor function depends on the sensitivity to CGRP [159]. Patients with migraine without aura are hypersensitive to CGRP because infusion of CGRP causes migraine or migraine-like headache in these patients [101]. A robust migraine response in FHM patients after CGRP would therefore indicate common migraine mechanism in FHM and MO.
Table 3: Number of patients and controls reporting headache and migraine headache

<table>
<thead>
<tr>
<th></th>
<th>FHM</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate headache (0-120 min)</td>
<td>5</td>
<td>7</td>
<td>0.65</td>
</tr>
<tr>
<td>Delayed headache (2-14h)</td>
<td>3</td>
<td>5</td>
<td>0.65</td>
</tr>
<tr>
<td>Migraine according to ICHD or migraine-like headache</td>
<td>2</td>
<td>1</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Groups compared with Fisher’s exact test.

We found no difference in the prevalence of migraine attacks fulfilling the IHS criteria for migraine with or without aura between FHM patients and controls (Table 3).

CGRP in a slightly larger dose (2 µg/min) induced delayed headache in 9 out of 9 MO patients [101] against only 3 out of 9 patients in our study, but also severe hypotension in 2 out of 12 patients which prompted us to use the lower dose of 1.5 µg/min that caused immediate headache in 50 % of healthy volunteers [121].

We found marked vascular effects of CGRP, but no differences in the vascular variables between FHM and controls could be found.

Animal studies have demonstrated that CGRP-induced vasodilation is insufficient to activate nociceptors [160], but we know from other studies that migraine patients have an arterial hypersensitivity to other migraine triggers [141].

This seems not to be the case for FHM patients, a finding corroborated by the larger AUCSTA in the control group. Therefore, we suggest that it is unlikely that FHM patients share the hypersensitivity to CGRP with migraine without aura.

Clinical significance of present findings
The phenotypical similarities and the great clinical overlap between FHM and common types of migraine [48] suggest common neurobiological pathways. We show here that the FHM genotype does not confer hypersensitivity to the known migraine trigger CGRP because activation of the CGRP - cyclic AMP pathways failed to induce more migraine aura or migraine headache in FHM pa-
tients than in healthy volunteers. We therefore suggest that neurobiological pathways responsible for migraine headache in MO and MA patients may be distinct from pathways responsible for migraine headache in FHM patients. Based on our results it seems unlikely that the new CGRP-antagonists would be effective in the management of FHM patients.

Electrophysiology and FHM (paper IV)

In the common forms of migraine, MA and MO, the brain and brain stem are characterized interictally by habituation deficits in the form of amplitude decrease of evoked responses or reflexes during repeated stimulation. This has been reported for visual, auditory, somatosensory and nociceptive evoked cortical potentials [122], and for the nociception-specific blink reflex (nBR) [123, 124]. The habituation deficit of the nBR and visual evoked potentials are correlated in the same patients which suggests a common underlying mechanism [126]. It has been debated whether this mechanism is neuronal hyper excitability [161, 162]. If neuronal hyper excitability were indeed the molecular pathology and possible source of migraine, it would be highly interesting to study the habituation of cortical and subcortical evoked responses in FHM patients, where the genotype has been linked to neuronal hyper excitability.

Methods

To optimize recruitment, we used portable devices to record most subjects with their agreement at their own homes. Within the same week, 9 out of 16 subjects were thus examined at their home, and the remaining patients at the clinical neurophysiology laboratory of Glostrup Hospital. The investigators who performed the electrophysiological recordings and analyses were blinded to diagnosis.

All patients and healthy volunteers underwent a study of pattern-reversal visual evoked potentials (VEP) auditory evoked cortical potentials (AEP) and nociception-specific blink reflexes (nBR) in a semi-randomized order starting with either VEP or AEP. For the VEP recordings, habituation was calculated as the percentage change of N1-P1 amplitude between the 1st and 6th block of averaging, for the AEP, habituation was calculated as the percentage N1-P2 amplitude change between 1st and 4th blocks and habituation of the R2 blink reflex was defined as the percentage change of the R2 AUC between the 1st and the 5th block of recordings.

Results

Visual Evoked Potentials (VEP): The difference in habituation was significant between healthy subjects and the total group of patients (median HV=5.02%, median FHM= -16.61%), (P=0.025).

Auditory evoked potentials (AEP): Intensity dependence slopes (IDAP) calculated on global and block averages, though slightly steeper in patients, were not significantly different between FHM and control subjects, or within the two FHM genotypes.

Nociception-specific blink reflexes (nBR): Mean perception thresholds tended to be higher in FHM subjects than in healthy volunteers (P=0.088) and mean pain thresholds were significantly higher in the FHM group than in the control group (P = 0.039). The mean area under the curve (AUC) of the 1st block of nBR averages did not differ significantly between groups of subjects, nor did nBR latencies, but over the subsequent blocks of 5 responses, the decrease, i.e. habituation, of the nBR AUC was markedly more pronounced in both FHM groups than in the control group. The amplitude change in the 5th block relative to the 1st block was on average −18.0% in controls (median −12.86%), compared to -51.7% in FHM-1 (median −64.32%, P = 0.15) and −54.8% in FHM-2 subjects (median=−51.17%, P = 0.02).
and FHM-2 patients might be distinct from MO and MA.

tal trait of the common types of migraine.
tivity to these migraine-provoking substances is a very fundamen-
the CGRP - cyclic AMP pathways failed to induce more migraine
Based on our data from paper III, we also suggest that the FHM
way, as characteristically seen in both MO and MA patients.
not confer hypersensitivity to activation of the NO-cGMP path-
earlier results from MO and MA: FHM-1 or FHM-2 mutations do
With regard to headache mechanisms, our results fail to confirm
Cortical spreading depression (CSD) is the probable pathophysi-
FHM genes. It is thus possible that FHM should be considered
This has implications for our understanding of the headache
mechanisms and questions whether FHM patients share neuro-
biological background with MO and MA. Any generalisation of
results from FHM to the typical migraines must, therefore, be
considered controversial.

Genotype – phenotype correlation?
FHM has been considered a monogenic disease but for specific
mutations, and even among individuals with the same primary
genetic lesion, great clinical variability is found [107]. This could
indicate additional genetic complexity in FHM, possibly caused by
the high prevalence of polymorphisms in migraine phenotype
modifying genes [163].
CACNA1A missense mutations have been associated with FHM-1
and nonsense or splice site mutations with episodic ataxia type 2
(EA2). These disorders may thus be seen as allelic disorders [40].
Spinocerebellar ataxia type 6 (SCA6), a dominant cerebellar de-
genenerative disorder, is caused by CAG repeat expansions in
CACNA1A [164].
Missense point mutations in CACNA1A, which typically cause
episodic conditions, may also cause progressive cerebellar syn-
drome indistinguishable from SCA6 [165], which in turn may have
episodic features as seen in EA2 and FHM [166]. Interestingly,
patients with distinct mutations in CACNA1A may show overlapp-
ing clinical features [167] which suggest that EA2, FHM, and
SCA6 might represent a clinical continuum [168, 169].
Some FHM-2 mutations are associated solely with FHM-2 [68]
while others also involves epilepsy as part of its phenotypic spec-
trum [170, 171] and also the FHM-3 mutations have been associ-
ated with epilepsy [72].
A simple genotype-phenotype correlation for FHM has not re-
vealed much and may be too simplistic [172]. The mutated genes
may be necessary but not sufficient to cause the FHM phenotype.
This could point towards a new concept of the importance of the
FHM genes. It is thus possible that FHM should be considered
more like a syndromic form of migraine, similar to the migraine
features observed in other neurological conditions like cerebral
autosomal dominant arteriopathy with subcortical infarcts and
leukoencephalopathy (CADASIL) [173] and myopathy, encephalo-
pathy, lactic acidosis and stroke-like episodes (MELAS). Severe
prolonged migrainous symptoms may be a characteristic feature
of MELAS [174] and in CADASIL, migraine with aura is a very fre-
quent symptom [175]. In one CADASIL family, typical FHM attacks
have been described [176].
It could therefore be speculated that the FHM phenotype, in
analogy with these conditions, might be considered secondary to
genetic changes per se, rather as a consequence of functional
effects of the mutations [177]. As a corollary it could be noted
that attacks of hemiplegic migraine may be seen in otherwise
migraine-free cystic fibrosis patients (with mutated CFTR chloride
channels) during cough episodes [178].

Treatment of FHM patients
FHM is a severe condition, with alarming aura symptoms but the
best acute migraine treatment, the triptans [179], are currently
contraindicated in FHM-patients [180] even though they are
probably effective also in FHM [181]. Mechanism-based headache
research has identified important migraine triggers and thereby
opened a new avenue for migraine treatment, because antago-
nism to these triggers constitutes effective migraine treatments
[92, 152]. The new CGRP-antagonists have no vascular effects
that could limit their use in FHM patients [182], and could poten-

Figure 23
The areas under the curve (AUC: μsec × msec) (mean ± standard error of the mean) in the 5 blocks of averages in the subject groups. The habituation is more pronounced in FHM patients than in healthy subjects (HV).
Initially be a major breakthrough in the management of FHM patients. Based on the works in this thesis, we suggest that FHM patients do not share pathophysiological pathways with MO and MA patients, because FHM patients are not hypersensitive to GTN and CGRP. It thus seems unlikely that antagonists to CGRP and NO would be effective in the management of FHM patients. Proper randomized controlled trials with these compounds are needed, to prove or disprove this prediction.

Methodological considerations
The more common types of migraine are not associated with any of the known FHM mutations [51-54], but non-penetrant mutation in some families may lead to healthy gene carriers. That could confuse the picture and might be interpreted as an involvement of the mutations in the more common forms of migraine [183]. The mutations in our patient material were not 100% penetrant, as healthy gene carriers were found in some of the families [48]. The most frequent FHM-1 mutation, the T666M mutation [105], was not represented in this study, but since there is a high clinical heterogeneity within [184] and between families with the T666M mutation [105], the results from our FHM-1 studies may be valid for the whole FHM-1 population.

Bearing in mind the nature of the experiments, the conclusion in this thesis should be interpreted with some caution for a number of reasons.
The studies were carried out on a limited number of subjects and without placebo arm. Given that FHM is a very rare disease with prevalence of approximately 0.006 % [185], and the number of well defined mutation carriers is even smaller [48], we decided to apply a group comparison design to avoid the risk of drop-outs in a cross-over design. It could be argued that the induced migraine-like headache is not specific for migraine mechanisms. Thus, both GTN and CGRP may induce neurovascular headache in healthy subjects [121, 127]. Other vasoactive substances also induce headache in healthy subjects [88, 89]. Nevertheless these studies [94, 101] clearly demonstrated that migraineurs are more sensitive than controls in terms of headache or migraine induction. Thus, the absence of robust headache or migraine-induction in patients with FHM indicates that FHM patients do not share the hypersensitivity to migraine-inducing substances known from MO and MA patients. Compared with the general population, FHM probands have a significantly increased risk of MA, which suggests that the genetic abnormality causing FHM may also cause attacks with the symptomatology of MA [50]. It could therefore be argued that it is more relevant to compare hypersensitivity to known migraine triggers between FHM and MA. In the GTN model of migraine, MA patients [119] exhibit similar or slightly smaller hypersensitivity than MO patients [95, 117]. CGRP causes migraine and migraine-like headache in MO [101], but the effect of CGRP in MA patients has not yet been studied.

As we hypothesised that FHM may be seen as a part of the migraine spectrum [186, 187], we would expect a similar response to known migraine triggers across subtypes. Despite these limitations, our experiments were carried out on a group of genetically well-defined patients, which strengthens the results and suggests that the FHM genotype does not confer hypersensitivity to known migraine triggers.

Can the results be dismissed because of a too low dosage of the provoking substances?

The vascular effects observed in study I and II are similar to data from earlier works [110, 127, 141] suggesting that GTN was indeed present in relevant doses in the subjects. During a migraine attack, CGRP in external jugular venous blood has been found increased 2-2.5 times compared with normal controls [28]. A recent study could however not confirm this finding [102]. In a study on healthy volunteers, infusion of 1.5 µg/min of h-αCGRP, (similar to the dose in study III), increased the plasma concentration approximately 3 to 4 times [121].

Future studies
Our material does not allow a separate evaluation of each mutation. It is therefore possible that some mutations could be associated with increased sensitivity to GTN and/or CGRP. Larger studies are needed to clarify this.

In a large proportion of FHM-patients, no mutations have been identified until now [48, 49]. These patients display a typical phenotype but without any associated mutations. This raises the question whether this group might share pathophysiological mechanisms with FHM or with the common types of migraine. Another way of dissecting migraine mechanisms could be to study subjects with sporadic hemiplegic migraine (SHM), a condition clinically indistinguishable from FHM, but without any affected family members [188]. The FHM mutations are rarely found in subjects with SHM [108, 189], but the similar hemiplegic phenotype suggests that FHM and SHM are pathophysologically related [186].

CONCLUSION
The aims of the present thesis were to test the hypothesis that FHM mutations might be associated with hypersensitivity to known migraine provoking substances and, thereby, share pathophysiological pathways with the common types of migraine, but our results disprove this hypothesis. Thus, FHM seems very different from MO and MA, both genetically and pathophysiological. The fact that FHM genes regulate ion homeostasis cannot be extrapolated to MO and MA, and our results do not support the assumption that all migraines are channelopathies or ionopathies.

SUMMARY
Familial hemiplegic migraine (FHM) is a rare, dominantly inherited subtype of migraine with aura, where hemiplegia occurs during the aura phase. Mutation screening of families with FHM has revealed a range of different mutations. The mutated FHM genes code for ion transport proteins. Animal and cellular studies have associated the mutated FHM genes with disturbed ion homeostasis, altered cellular excitability and altered neurotransmitter release. Abnormal cortical excitability due to dysfunctional ion-channels might facilitate cortical spreading depression (CSD) and thereby migraine aura and migraine headache. Genotyped FHM patients offer us the chance to study the interplay between genotype and phenotype and may be regarded as a genetic migraine model. FHM studies might open for a better understanding of the molecular migraine pathology, and potentially help to unravel the pathogenesis of the more common migraine forms. We have therefore studied genotyped FHM patients to understand the effect of genotype on the response to migraine provoking substances. We show here that two known migraine triggers failed to induce more migraine aura or migraine headache in FHM-patients than in healthy controls, thus indicating that the FHM genotype does not confer hypersensitivity to these migraine triggers. This has
implications for our understanding of the headache mechanisms and raises the question whether FHM share neurobiological background with the common types of migraine. The aims of the present thesis were to test the hypothesis that FHM mutations might be associated with hypersensitivity to known migraine triggers and, thereby, share pathophysiological pathways with the common types of migraine, but our results disprove this hypothesis. Thus, FHM seems very different from MO and MA, both genetically and pathophysiologically. The fact that FHM genes regulate ion homeostasis cannot be extrapolated to the common types of migraine.

Acknowledgement
I would like to thank all participating FHM patients – I hope this work will inspire and spark further works and discussions on FHM pathogenesis, diagnosis and treatment.

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