Familial hemiplegic migraine
– an experimental genetic headache model

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The experiments included in the present PhD thesis were performed during my appointment as research fellow from 2004 to 2008 at the Danish Headache Centre and Department of Neurology at Glostrup Hospital and the University of Copenhagen.

My supervisors were associate professor Messoud Ashina, MD, DMSc, PhD and Professor Jes Olesen, MD, DMSc.

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- Bolla M, Hansen JM, Thomsen LL, Ashina M, Olesen J, Schoenen J: The electrophysiological profile of genotyped patients with familial hemiplegic migraine. *In preparation*
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>FHM-1</td>
<td>Familial hemiplegic migraine of type 1</td>
</tr>
<tr>
<td>FHM-2</td>
<td>Familial hemiplegic migraine of type 2</td>
</tr>
<tr>
<td>FHM-3</td>
<td>Familial hemiplegic migraine of type 3</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl trinitrate</td>
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<tr>
<td>CGRP</td>
<td>Calcitonin gene related peptide</td>
</tr>
<tr>
<td>MA</td>
<td>Migraine with aura</td>
</tr>
<tr>
<td>MO</td>
<td>Migraine without aura</td>
</tr>
<tr>
<td>CSD</td>
<td>Cortical spreading depression</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>PDE</td>
<td>Phosphodiesterase</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>STA</td>
<td>Superficial temporal artery</td>
</tr>
<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>VEP</td>
<td>Visual evoked potentials</td>
</tr>
<tr>
<td>IDAP</td>
<td>Intensity dependence of the auditory evoked potential</td>
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<tr>
<td>nBR</td>
<td>Nociceptive blink reflex</td>
</tr>
<tr>
<td>HV</td>
<td>Healthy volunteer</td>
</tr>
<tr>
<td>VRS</td>
<td>Verbal rating scale</td>
</tr>
<tr>
<td>EA2</td>
<td>Episodic ataxia type 2</td>
</tr>
<tr>
<td>SCA6</td>
<td>Spino cerebellar ataxia type 6</td>
</tr>
<tr>
<td>CADASIL</td>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy</td>
</tr>
<tr>
<td>MELAS</td>
<td>Myopathy, encephalopathy, lactic acidosis and stroke-like episodes</td>
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<tr>
<td>SHM</td>
<td>Sporadic hemiplegic migraine</td>
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Chapter one

Introduction and aims of this thesis

Familial hemiplegic migraine (FHM) is a rare subtype of migraine with transient hemiplegia during the aura phase. FHM is dominantly inherited, and mutation screening of families with FHM has revealed a range of different mutations associated with the FHM phenotype. 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. 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 aura (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 similarities between FHM, MA and MO. This thesis is based on works that examine and describe the potential influence of the FHM genotype on the response to known migraine provoking substances.

- Study 1 is a controlled study on the migraine inducing effect of nitric oxide in patients with familial hemiplegic migraine and mutated CACNA1A calcium channels (FHM-1).

- Study 2 is a controlled study on the migraine inducing effect of nitric oxide in patients with familial hemiplegic migraine and mutated 1A2 potassium-sodium pumps (FHM-2).

- Study 3 is a controlled study on the migraine inducing effect of calcitonine gene related peptide (CGRP) in genotyped patients with FHM-1 and FHM2.

- Study 4 is a controlled electrophysiological study in genotyped FHM-1 and FHM-2 patients, to explore the impact of FHM-mutations on brain electrophysiology.
Chapter two

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\(^11\). 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 have been considered important in the development of migraine pain\(^17\), and vasodilatator mechanisms and neuropeptides became therefore a focus in headache research\(^18\) giving rise to the pathophysiologic 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 intra cranial 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 \textit{neurovascular headache} \(^{27}\), 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 \(^{28,29}\).

\textbf{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 \(^{30}\).}
Between attacks, migraine patients are totally symptom-free, suggesting that the underlying brain dysfunction is a periodic event. Other episodic neurological diseases have been associated with changes in ion channel function, including muscle diseases \(^{31, 32}\) and ataxia \(^{33}\). Epilepsy is another paroxysmal neurological disorder with temporary dysfunction of the cerebral cortex during attacks, associated with ion channel dysfunction \(^{34}\). These channelopathies show paroxysmal attacks precipitated by physiological stress or other triggers.

The balance between excitatory and inhibitory signals on neuronal excitability is quite delicate, and even relatively small alterations in channel activity may tip this balance. It has been speculated that abnormal cortical excitability due to dysfunctional ion channels may trigger migraine attacks \(^{35}\).

It seems reasonable to suspect that migraine aura, caused by cortical spreading depression \(^{36, 37}\), is also consistent with a channelopathy, given the transient nature of the symptoms.

The notion that migraine might be a channelopathy has prompted the search for a possible genetic basis for migraine. Due to both clinical and genetic heterogeneity in migraine patients, as well as a long list of possible environmental causes and trigger factors, no decisive genetic factors have yet been identified for the common types of migraine.
4.1 FHM 1, FHM 2, FHM 3 and others

In 1873, Liveing first reported the occurrence of transient weakness during otherwise typical migraine episodes, but it was not until 1953 that Whitty distinguished the sporadic and familial forms of hemiplegic migraine. FHM is phenotypically characterized by fully reversible half-sided weakness and other aura symptoms preceding or accompanying a migrainous headache.

In most large FHM families studied, FHM is inherited in an autosomal dominant manner. Mutation screening of families with FHM has revealed a range of different mutations associated with the FHM phenotype.

Familial hemiplegic migraine of type 1: FHM type 1 (FHM-1) is associated with missense mutations in the CACNA1A gene on chromosome 19p13, encoding the α1A subunit of calcium channels.

Figure 2: Secondary structure of the CaV2.1 α1 subunit and location of the familial hemiplegic migraine 1 mutations identified so far. In black: mutations whose functional consequences have been studied in heterologous expression systems. Underlined: mutations whose functional consequences have been studied also in transfected neurons from CaV2.1−/− mice, adapted from 6.
and at least eighteen different missense mutations have been identified. This gene encodes the pore-forming $\alpha_{1A}$ subunit of the $\text{Ca}_{v}2.1$ (P/Q-type) voltage-gated neuronal calcium channel, which modulate release of neurotransmitters at peripheral and central synapses.

**Familial hemiplegic migraine of type 2:** FHM type 2 (FHM-2) is associated with mutations in the ATP1A2 gene encoding the $\alpha_2$ subunit of a $\text{Na}^+, \text{K}^+$-ATPase, and more than 20 mutations have been identified.

*Figure 3: Secondary structure of the $\text{Na}^+, \text{K}^+$-ATPase $\alpha$, subunit and location of the familial hemiplegic migraine 2 mutations identified so far. In black: mutations whose functional consequences have been studied in heterologous expression systems, adapted from.*
Familial hemiplegic migraine of type 3: FHM type 3 (FHM-3) is associated with mutations in the SCN1A gene on chromosome 2q24. The SCN1A gene encodes the α1 subunit of the neuronal voltage-gated sodium (Na_\text{v}1.1) channels.

Other subtypes of familial hemiplegic migraine
In a large proportion of FHM-patients, no mutations have been identified until now.

4.2 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), and many FHM-patients have MA and/or MO. However, it has been reported that MA and MO are not associated with any of the known FHM mutations. 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.

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.

4.3 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.

**FHM-1:** Calcium channels of the P/Q-type are found throughout the brain and central nervous system (CNS), with a high concentration of the \( \alpha_{1A} \) subunit in the cerebellum. 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. Other calcium channels are also found within the CNS and \( \text{Ca}^{2+} \) channels of both P/Q-, N-, and R-type control glutamate release. However, the \( \text{Ca}^{2+} \) influx through the P/Q-type channels trigger neurotransmitter release more effectively than \( \text{Ca}^{2+} \) influx through N- or R-type channels, possibly because the P/Q-type channels are located closer to the glutamate release sites. 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.
In human neurons, FHM-1 mutations increase single-channel Ca\(^{2+}\) influx, whereas in human cerebellar granule cells a decrease in the density of functional calcium channels is found in the membrane\(^{63}\).

The net phenotype of the FHM-1 mutations have thus been suggested to be one of gain-of function for most mutations\(^{62}\) because the combination of a lower activation threshold and the increase in single-channel opening probability may lead to an increased Ca\(^{2+}\) 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\(^{64}\).

**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 \(\alpha_2\) subunit of the Na\(^+\),K\(^+\)-ATPase\(^6\). The molecular effects of the FHM-2 mutations range from reduction of Na\(^+\),K\(^+\)-ATPase activity\(^{45,65-69}\), abnormally functioning channels\(^{70}\), to expression of fully functional but kinetically changed channels\(^{71,72}\). 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 \(\alpha_2\) Na\(^+\),K\(^+\)-ATPase, which has been termed *functional haploinsufficiency*\(^{45}\).

**FHM-3:** The mutated gene product in FHM-3, the Na\(_V\) 1.1 channel is expressed in cortical neurons\(^{73}\), 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\(^{74,75}\).

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 47.

The mutated Na, 1.1 channel has been associated with a pronounced but self-limiting neuronal hyper excitability but in some cases also to hypo excitability 76. 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 77.

4.4 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 hypo perfusion 78, similar to migraine with aura 79, which suggest that CSD is the most likely mechanism of hemiplegic aura 80, 81. Experiments in rats show that CSD in both the cortex 82 and hippocampus 83 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 84.

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 64, 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 86, giving weight to the hypothesis that genetic predisposition, CSD and migraine might be linked 64. Based on this it seems likely that FHM-1 patients could be more susceptible to aura and headache.
The functional haploinsufficiency of FHM-2 mutations may reduce the electrochemical Na⁺ gradient required to drive the astrocytic glutamate transporters, which is co-localized with the astro-glial α₂ isotype Na⁺, K⁺-ATPase. This would reduce the removal of glutamate and lower the threshold for cortical spreading depression. 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.

4.5 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.
More mutations have been identified in several families \(^{89}\) 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 \(^{6,7,35}\). 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.
In vitro studies have contributed in the characterization of receptors in cranial blood vessels and the identification of new possible antimigraine agents. 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.

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

5.1 Nitric oxide and cGMP

![Figure 6: The nitric oxide – cGMP pathway, adapted from](image-url)
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 \(^{97,98}\).

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 \(^{99}\). A good example is sildenafil (Viagra\textsuperscript{TM}), 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 \(^{100}\).

5.2 CGRP and cAMP
Calcitonine-gene related peptide (CGRP) is a 37-amino acid neuropeptide \(^{101}\) that activates adenylyl cyclase, thereby increasing cyclic adenosine monophosphate (cAMP) levels \(^{102,103}\). CGRP is involved in migraine pathogenesis \(^{104}\), and might be released from the cranial circulation during migraine attacks \(^{28,105}\).

\(\text{CGRP} \rightarrow \text{CRLR} \rightarrow \text{G} \alpha \gamma \rightarrow \text{RCP} \rightarrow \text{Adenyl Cyclase} \rightarrow \text{cAMP}\)

\(\text{Figure 7: The CGRP receptor complex is proposed to comprise a ligand binding protein (CRLR), an accessory protein (RAMP1), and an accessory protein for coupling to cellular signal transduction pathways (RCP), adapted from}^{106}\).\)
CGRP activates adenylyl cyclase thereby increasing cAMP levels\textsuperscript{102,103}. 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\textsuperscript{107}. The migraine inducing effect of CGRP can thus be attributed to hypersensitive to activation of the CGRP – cyclic AMP pathways\textsuperscript{91}. 
Chapter six

In Vivo Functional Studies of FHM mutations in patients

6.1 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. 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. In total 33 subjects from the Danish cohort have a known mutation, and were thus eligible for participation in these studies.

Study 1: 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. 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), was not present in any
of the participating patients, but R583Q, the second most frequent mutation\textsuperscript{112,113} and the most prevalent Danish FHM-1 mutation\textsuperscript{50} was represented.

**Study II:** The Danish cohort consisted of 13 FHM-2 patients with known mutations\textsuperscript{50}. 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\textsuperscript{50}, 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)), 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.

6.2 **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. The participant then rested for 30 min before baseline measurements of blood pressure, heart rate and ECG were done and the infusion started, using a time and volume controlled infusion pump (Braun Perfusor, Melsungen, Germany). Headache intensity, middle cerebral artery mean blood flow velocity ($V_{\text{meanMCA}}$), superficial temporal artery diameter, end-tidal partial pressure of CO2, adverse events and vital signs were recorded at T-10, and then every 10 min until 120 min after start of infusion.

Exclusion criteria for the patients were: Any daily medication apart from oral contraceptives; serious somatic or psychiatric diseases.

The control subjects did not have a history of migraine or any other type of headache (except tension type headache less than once a month). The intake of coffee, tea, cocoa or other methylxanthine containing foods or beverages was not allowed for the last 8 h before the start of the study to avoid a possible affection of the cerebral blood flow.

All studies were approved by the Ethics Committee of the County of Copenhagen and undertaken in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000. All subjects gave informed consent to participate in the study.

6.3 Headache intensity

Headache intensity was recorded on a verbal rating scale (VRS) from 0 to 10. The subjects were discharged from the hospital two hours after start.
of the infusion, and asked to complete a headache diary every hour until
14 h after start of the experiments. The diary included headache character-
istics and accompanying symptoms according to the International Headache
Society⁵, any rescue medication taken and adverse events. Subjects were
allowed to take rescue medication of their own choice at any time.

6.4 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 hemi-
sphere. From its origin, the MCA extends laterally and horizontally in the
lateral cerebral fissure.

CBF is unchanged by GTN¹¹⁴ and CGRP²³, and a reduction in middle
cerebral artery blood flow velocity will therefore indicate a dilation of the
middle cerebral artery. The mean maximal blood velocity in the middle
cerebral artery (VmeanMCA) was recorded bilaterally by transcranial Doppler
(TCD) with hand-held 2MHz probes (Multidop X, DWL, Sipplingen,
Germany). We used the transtemporal approach, whereby the middle
cerebral artery can be visualized.

Figure 8: Transtemporal Doppler measurements of the middle cerebral artery (MCA),
adapted from¹¹⁵.
Fixed probes were not used since they may cause discomfort and even headache\textsuperscript{116}. A time-averaged mean over 4 seconds or approximately 4 cardiac cycles was used as final measure for each time point. A fixed point for measurements of $V_{\text{mca}}$ was chosen along the MCA as the point that was as close as possible to the bifurcation between the MCA and the anterior cerebral artery. 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\textsuperscript{117} 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\textsuperscript{119}, but we opted for the present set-up, because it allowed recordings from both the intracranial and extra cranial arteries.

\textit{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)\textsuperscript{120}. 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.

### 6.5 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. The primary endpoints were differences in incidence of migraine and migraine-
like headache and in the AUC for headache score ($\text{AUC}_{\text{headache} 0-120 \text{ min}}$ and $\text{AUC}_{\text{headache} 2 \text{ h-14 h}}$), $V_{\text{meanMCA}}$ ($\text{AUC}_{V_{\text{meanMCA}}}$), STA ($\text{AUC}_{\text{STA}}$) 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 in approximately 80% of FHM-1 patients as reported previously in common types of migraine\textsuperscript{98,122} and migraine like headache in less than 10% of healthy controls\textsuperscript{123,124}. We estimated that 8 subjects should be included in each group\textsuperscript{125}.

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\textsuperscript{104} and migraine-like headache in less than 10% of healthy controls\textsuperscript{126}. We estimated that 9 subjects should be included in each group\textsuperscript{125}.

6.6 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\textsuperscript{127}, and for the nociception-specific blink reflex (nBR)\textsuperscript{128,129}. The nociceptive blink reflex (nBR) is mediated by brainstem neurons\textsuperscript{130}, and lack of habituation of the this reflex is a reproducible abnormality found in migraineurs between attacks in evoked potential studies\textsuperscript{131} and also in healthy volunteers with a family history of migraine\textsuperscript{129}. Deficient nBR habituation might thus be a traitmarker 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\textsuperscript{4}. 
Chapter seven

Results

7.1 Nitric oxide and FHM-1 (Paper I)
Nitric oxide is an effective and reproducible trigger of headache and migraine in migraine patients. We therefore used the GTN migraine provocation model to explore the functional consequences of the R583Q and C1369Y gene mutations in FHM-1 patients. Activation of the NO–cGMP pathway failed to induce migraine aura or migraine headache in patients with FHM-1. This finding is in sharp contrast to results in migraine patients with and without aura, where GTN induces migraine in 50–80% of patients.

GTN failed to induce a migraine aura
Cortical spreading depression (CSD), discovered by Leão, has been linked to migraine aura pathogenesis in both observational, animal and human studies. Cerebral blood flow (CBF) studies during hemiplegic aura showed a spreading cortical hypo perfusion, similar to migraine with aura, which suggest that CSD is the most likely mechanism of hemiplegic aura. 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. It has been proposed that CACNA1A mutations leads to increased release of glutamate, and an increased susceptibility to CSD.

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, and nitric oxide has also been linked to the modulation of the calcium entry through P/Q type calcium channels, and the transduction between neuronal activity and increased CBF after CSD. Furthermore, Read and colleagues showed that GTN stimulates the release of nitric oxide in response to CSD and CSD increases the levels of cGMP in the cortex and brain stem. 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. 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, and in a study of 22 patients, sublingually applied GTN induced aura in 3 patients.

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 R5583Q and C1369Y mutations. 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 and maintaining migraine headache. Interestingly, the study by Christiansen et al. 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

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<th>FHM-1</th>
<th>Controls</th>
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</tr>
<tr>
<td>Migraine according to ICHD</td>
<td>1</td>
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<td>0.47</td>
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Groups compared with Fisher’s exact test.

*Table 1: Number of patients and controls reporting headache and migraine headache, adapted from Hansen et Al.*

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aura symptoms, a similar headache phase and similar associated symptoms\textsuperscript{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\textsuperscript{122, 133}. GTN have also been reported to induce a more pronounced headache response in migraine patients than in healthy controls\textsuperscript{97, 124}.

The present results therefore suggest that the R583Q / C1369Y mutations do not cause hypersensitivity to GTN and consequently seem to affect neurobiological pathways other than those in MA and MO. Two out of 8 FHM-1 patients had both FHM-1 and MA and only one of these, with the R583Q mutation, developed delayed headache fulfilling the criteria for migraine without aura. This is similar to the placebo rate of migraine induction in a study, where 1 out of 10 MO patients developed a migraine

![Figure 12: Individual and median headache scores on a verbal rating scale (VRS) during immediate (0–120 min) and delayed phases (2–14 h) after start of the GTN infusion in 8 patients with FHM-1 and 9 controls. There were significantly higher pain responses during the immediate phase in the patient group compared to the control group (P = 0.008). There were no difference between patients and controls in the 14 hours following the GTN infusion (P = 0.167). Thick lines in figure are median pain scores, adapted from Hansen et Al\textsuperscript{1}.

FAMILIAL HEMIPLEGIC MIGRAINE
attack after placebo. Interestingly, family members (n=5) of this patient with the same mutation but without known co-existing common types of migraine did not develop migraine. In the light of these surprising findings, one might suggest that neurobiological pathways of co-existing migraine with aura are sometimes distinct from pathways involved in FHM-1.

In line with previous studies on migraine patients, the FHM-1 patients developed more immediate headache (0–2 h) than controls. Arterial dilatation may cause headache, and GTN infusion causes a more pronounced dilation of extra- and intracerebral arteries in migraine patients than in controls. We found changes in the diameter of the STA, and a decrease in the $V_{\text{meanMCA}}$ comparable to earlier studies using the same dose of GTN, but we did not detect any differences in $V_{\text{meanMCA}}$, 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. 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 immediate headache in the control group, however, is similar to a large study by Sances et al. Studies on the R593Q-mutation also examined in the present study showed that FHM mutated human CaV2.1 channels display an increased open probability, thus allowing FHM-1 channels to carry larger Ca\(^{2+}\) fluxes than in the wild type. 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 neuropathic pain states. 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.

### 7.2 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 \(\alpha_2\) Na\(^+\),K\(^+\)-ATPase, which has been termed functional haploinsufficiency. Reduced activity of the Na\(^+\),K\(^+\)-ATPase may reduce the removal of glutamate and lower the threshold for cortical spreading depression (CSD). Pharmacological inactivation of the Na\(^+\)K\(^+\)-ATPase (similar to the effects of FHM-2 mutations) causes CSD-like depolarization and...
stimulation of the NO – cGMP pathway has been shown to inhibit Na+K+-ATPase activity. 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 or never 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 can therefore not entirely rule out the possibility, that the reported attack was in fact a spontaneous one. Another possibility is that this particular mutation is associated with hypersensitivity of the NO-cGMP pathway. To clarify whether the hemiplegic aura could be triggered again, we could have repeated the GTN-infusion as previously described in migraine with typical aura, 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. 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.

Surprisingly, we found no differences in the prevalence of migraine attacks fulfilling the IHS criteria between FHM-2 and controls (table 2). GTN

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<th>FHM-1</th>
<th>Controls</th>
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<td>Immediate headache (0–120 min)</td>
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<td>0.65</td>
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<tr>
<td>Delayed headache (2–14h)</td>
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<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.

Table 2: Number of patients and controls reporting headache and migraine headache, adapted from Hansen et Al.
infusion induced migraine in only 25% of FHM-2 patients, which is much lower than what is seen in both MO and MA.

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\textsuperscript{153}, 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

![Figure 14: Headache scores on a verbal rating scale (VRS) during immediate (0–120 min) and delayed phases (2–14 h) after start of the glyceryl trinitrate (GTN) infusion in eight patients with familial hemiplegic migraine type 2 (FHM-2) and nine controls. There was no difference in the AUC between patients and controls during immediate (P = 0.37) and delayed phases (P = 0.09) following the GTN infusion. Thick lines in figure are median pain scores, adapted from Hansen et Al\textsuperscript{2}.](image-url)
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\textsuperscript{104}, which has never been examined in FHM patients.

### 7.3 CGRP and FHM (Paper III)

Calcitonin gene-related peptide (CGRP) is a neuropeptide\textsuperscript{101} that is present in structures relevant to migraine and its release dilates cephalic arteries\textsuperscript{28, 29, 154-156}. 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\textsuperscript{104} and a mild headache in healthy controls\textsuperscript{126}. Even more importantly, CGRP-receptor antagonism has documented efficacy in the treatment of migraine attacks\textsuperscript{157}.

An orally available CGRP antagonist have been tested in randomized clinical trials and have been found both safe and efficient\textsuperscript{95, 158}.

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 GGRP, similar to that observed in patients with MO\textsuperscript{104}. 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 \(^{80}\), and a model has been proposed that links FHM mutations with a propensity to CSD \(^{64}\). Animal studies have established a link between FHM-1 mutations and increased susceptibility to CSD \(^{86}\), 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 \(^{159}\), probably via stimulation of phospholipase C \(^{160}\). CGRP acts in part via the same mechanism \(^{161}\).

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 \(^{162}\) which has been linked to migraine pathogenesis \(^{163}\). Sensitivity to CGRP may be a determinant of the nociceptive threshold because nociceptor function depends on the sensitivity to CGRP \(^{164}\).

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<th>Controls</th>
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<tr>
<td>Immediate headache (0–120 min)</td>
<td>5</td>
<td>7</td>
<td>0.65</td>
</tr>
<tr>
<td>Delayed headache (2–h–14h)</td>
<td>3</td>
<td>5</td>
<td>0.65</td>
</tr>
<tr>
<td>Migraine according to ICHD or migraine-like headache</td>
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<td>0.58</td>
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Groups compared with Fisher’s exact test.

*Table 3: Number of patients and controls reporting headache and migraine headache, adapted from Hansen et Al \(^{3}\)*
Patients with migraine without aura are hypersensitive to CGRP because infusion of CGRP causes migraine or migraine-like headache in these patients\(^{104}\). A robust migraine response in FHM patients after CGRP would therefore indicate common migraine mechanism in FHM and MO.

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\(^{104}\) 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\(^{126}\).

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**Figure 16**: Headache scores on a verbal rating scale (VRS) during immediate (0–120 minutes), and delayed phases (2–14 h) after start of the CGRP infusion in 9 patients with FHM and 10 controls. The AUC_{headache} 0–120 min did not differ between patients, 10 (0–142.5), and controls, 30 (0–91.25), (P = 0.661). We found no difference in the AUC_{headache} 2 h–14 h between patients, 0 (0–9), and controls, 0.25 (0–3.13), (P =1.00). Thick lines in figure are median pain scores, adapted from Hansen et Al\(^{3}\).
We found marked vascular effects of CGRP, but no differences in the vascular variables between FHM and controls could be found (Figures 17a and b).

Animal studies have demonstrated that CGRP-induced vasodilatation is insufficient to activate nociceptors, but we know from other studies that migraine patients have an arterial hypersensitivity to other migraine triggers. 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 suggest common neurobiological pathways. We show here that the FHM genotype does not confer hypersensitiv-
ity 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 patients 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.

7.4 Electrophysiology and FHM (paper IV)
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 127, and for the nociception-specific blink reflex (nBR) 128,129. The habituation deficit of the nBR and visual evoked potentials are correlated in the same patients which suggests a common underlying mechanism 131. It has been debated whether this mechanism is neuronal hyper excitability 166, 167. 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).

**Comments**

If neuronal hyper excitability was the culprit for the interictal habituation deficit of evoked responses in the common forms of migraine, and if the latter and familial hemiplegic migraine (FHM) belonged to the same patho-
physiological spectrum, one would expect that FHM patients might present at least some of the electrophysiological abnormalities found in migraine with/without aura. The results presented here provide little support for this hypothesis.

Contrary to the common forms of migraine, FHM is not characterized by a deficient, but rather by an increased habituation in cortical/brain stem evoked activities. Although these results need to be confirmed, our results suggest that the pathophysiology differs between FHM, MO and MA.
Chapter eight
The significance of the present results

8.1 FHM and common types of migraine – do they share a common pathway?
Cortical spreading depression (CSD) is the probable pathophysiological mechanism behind migraine aura, in both MA and FHM. Even though animal studies have linked FHM-1 mutations to an increased susceptibility to CSD, our results show that known migraine inducing substances were not able to induce migraine aura in FHM patients. This is similar to what is found in MA patients. The aura mechanisms in MA and FHM are thus probably very similar.

With regard to headache mechanisms, our results fail to confirm earlier results from MO and MA: FHM-1 or FHM-2 mutations do not confer hypersensitivity to activation of the NO-cGMP pathway, as characteristically seen in both MO and MA patients. Based on our data from paper III, we also suggest that the FHM genotype is probably not very important for the hypersensitivity to the migraine provoking peptide, CGRP, because activation of the CGRP–cyclic AMP pathways failed to induce more migraine headache in FHM patients than in healthy volunteers. Hypersensitivity to these migraine-provoking substances is a very fundamental trait of the common types of migraine.

Based on the present results, we suggest that neurobiological pathways responsible for triggering migraine headache in FHM-1 and FHM-2 patients might be distinct from MO and MA.

This has implications for our understanding of the headache mechanisms and questions whether FHM patients share neurobiological background with MO and MA. Any generalisation of results from FHM to the typical migraines must, therefore, be considered controversial.
8.2 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 \(^{111}\). This could indicate additional genetic complexity in FHM, possibly caused by the high prevalence of polymorphisms in migraine phenotype modifying genes \(^{168}\).

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 \(^{41}\). Spinocerebellar ataxia type 6 (SCA6), a dominant cerebellar degenerative disorder, is caused by CAG repeat expansions in CACNA1A \(^{169}\).

Missense point mutations in CACNA1A, which typically cause episodic conditions, may also cause progressive cerebellar syndrome indistinguishable from SCA6 \(^{170}\), which in turn may have episodic features as seen in EA2 and FHM \(^{171}\). Interestingly, patients with distinct mutations in CACNA1A may show overlapping clinical features \(^{172}\) which suggest that EA2, FHM, and SCA6 might represent a clinical continuum \(^{173},^{174}\).

Some FHM-2 mutations are associated solely with FHM-2 \(^{70}\) while others also involves epilepsy as part of its phenotypic spectrum \(^{175},^{176}\) and also the FHM-3 mutations have been associated with epilepsy \(^{74}\).

A simple genotype-phenotype correlation for FHM has not revealed much and may be too simplistic \(^{177}\). 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) \(^{178}\) and myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). Severe prolonged migrainous symptoms may be a characteristic feature of MELAS \(^{179}\) and in CADASIL, migraine with aura is a very frequent symptom \(^{180}\). In one CADASIL family, typical FHM attacks have been described \(^{181}\).
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. 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.

8.3 Treatment of FHM patients
FHM is a severe condition, with alarming aura symptoms but the best acute migraine treatment, the triptans, are currently contraindicated in FHM-patients even though they are probably effective also in FHM. Mechanism-based headache research has identified important migraine triggers and thereby opened a new avenue for migraine treatment, because antagonism to these triggers constitutes effective migraine treatments.

The new CGRP-antagonists have no vascular effects that could limit their use in FHM patients, and could potentially 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.

8.4 Methodological considerations
The more common types of migraine are not associated with any of the known FHM mutations, 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. The mutations in our patient material were not 100% penetrant, as healthy gene carriers were found in some of the families.

The most frequent FHM-1 mutation, the T666M mutation, was not represented in this study, but since there is a high clinical heterogeneity within
and between families with the T666M mutation, 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%, and the number of well defined mutation carriers is even smaller, 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. Other vasoactive substances also induce headache in healthy subjects. Nevertheless these studies 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. 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 exhibit similar or slightly smaller hypersensitivity than MO patients. CGRP causes migraine and migraine-like headache in MO, 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, 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 sug-
gests 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 \textsuperscript{114, 132, 146} 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 \textsuperscript{28}. A recent study could however not confirm this finding \textsuperscript{105}. 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 \textsuperscript{126}.

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 \textsuperscript{50, 51}. 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 \textsuperscript{192}. The FHM mutations are rarely found in subjects with SHM \textsuperscript{112, 193}, but the similar hemiplegic phenotype suggests that FHM and SHM are pathophysiologically related \textsuperscript{190}.

8.5 Conclusion
The aims of the present thesis were to test the hypothesis that FHM mutations might be associated with hypersensitivity to known migraine provok-
ing 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 pathophysiologically. 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.
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.
Migræne er en hyppig sygdom, som rammer omkring 12 % af befolkningen i den vestlige verden. Det er endnu ikke lykket at identificere gener der disponerer til typisk migræne. Anderledes forholder det sig med familier hemiplegisk migræne (FHM), eller migræne med lammelser, som er en sjælden, dominant arvelig subtype af migræne med aura, hvor ét af aurafænomenerne er halvsidige lammelser. Genomscreeninger i FHM-familier har afsløret en række forskellige mutationer i gener der koder for iontransportører. Dyreeksperimenter og cellulære studier har koblet de muterede gener med ændringer i ionhomeostase, forandringer i både cellular ekstabilitet og frigivelse af neurotransmittorer. Ændret kortikal ekstabilitet forårsaget af dysfunktioner i ionkanaler kan tænkes at facilitere cortical spreading depression og dermed migræneaura og migrænehovedpine.

Genotypede FHM-patienter giver således mulighed for at undersøge sammenhængen mellem arveanlæg (genotype) og sygdomsbillede (fænotype). Funktionelle undersøgelser af FHM-patienter kan dermed virke som en genetisk migrænemodel. Sådanne studier kan åbne op for en bedre forståelse af den molekylære migrænepatofysiologi, og kan derved også give os viden om de mere almindelige migræntyper.

Tidligere undersøgelser har vist at visse signalstoffer kan inducere migræne hos migrænepatienter, og at denne følsomhed for migræneudløsende stoffer er en vigtig del af migrænenes basale patofysiologi.

Målet med afhandlingen var at klargøre om FHM-genotypen er forbundet med øget følsomhed for kendte migræneudløsende stoffer og således om FHM deler neurobiologisk baggrund med de almindelige migræntyper. Undersøgelserne viste at kendte migræneudløsende stoffer ikke inducerede mere migræneaura eller migrænehovedpine hos FHM-patienter. Dette
tyder på, at FHM-genotypen ikke medfører en øget følsomhed for disse migræneudløsende stoffer.

Disse resultater har betydning for vores forståelse af generelle hovedpinemekanismer, og stiller spørgsmål ved om FHM deler neurobiologi med de almindelige migrænetyper. Resultatet af disse undersøgelser tyder på at dette ikke er tilfældet. FHM adskiller sig således formodentlig fra de almindelige migrænetyper, både med hensyn til genetik og patofysiologi.
References


35. Pietrobon D. Calcium channels and channelopathies of the central nervous system. Mol Neurobiol. 2002;25:31-50
41. Ophoff RA, Terwindt GM, Vergouwe MN et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell. 1996;87:543-552


52. Thomsen LL, Olesen J, Russell MB. Increased risk of migraine with typical aura in probands with familial hemiplegic migraine and their relatives. Eur J Neurol. 2003;10:421-427


55. Jen JC, Kim GW, Dudding KA, Baloh RW. No mutations in CACNA1A and ATP1A2 in probands with common types of migraine. Arch Neurol. 2004;61:926-928


60. Starr TV, Prystay W, Snutch TP. Primary structure of a calcium channel that is highly expressed in the rat cerebellum. Proc Natl Acad Sci U S A. 1991;88:5621-5625


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


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


100. Kruuse C, Thomsen LL, Birk S, Olesen J. Migraine can be induced by sildenafil without changes in middle cerebral artery diameter. Brain. 2003;126:241-247


104. Lassen LH, Haderslev PA, Jacobsen VB et al. CGRP may play a causative role in migraine. Cephalalgia. 2002;22:54-61


118. Katz ML. Transcranial Color Doppler and transcranial Color Doppler Imaging. Online CME Courses: GE Healthcare


134. Leao AAP. SPREADING DEPRESSION OF ACTIVITY IN THE CEREBRAL CORTEX. J Neurophysiol. 1944;7:359-390


142. Read SJ, Hirst WD, Upton N, Parsons AA. Cortical spreading depression produces increased cGMP levels in cortex and brain stem that is inhibited by tonabersat (SB-220453) but not sumatriptan. Brain Res. 2001;891:69-77


146. Thomsen LL, Iversen HK, Brinck TA, Olesen J. Arterial supersensitivity to nitric oxide (nitroglycerin) in migraine sufferers. Cephalalgia. 1993;13:395-399; discussion 376


149. Luvisetto S, Marinelli S, Panasiti MS et al. Pain sensitivity in mice lacking the Ca(v)2.1alpha(1) subunit of P/Q-type Ca(2+) channels. Neuroscience. 2006;142:823-832


152. Sato T, Kamata Y, Irifune M, Nishikawa T. Inhibition of purified (Na+,K+)ATPase activity from porcine cerebral cortex by NO generating drugs. Brain Res. 1995;704:117-120


163. Dalkara T, Zervas NT, Moskowitz MA. From spreading depression to the trigemino-vascular system. Neurol Sci. 2006;27 Suppl 2:S86-90


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


Appendices: Papers 1-4
Familial hemiplegic migraine type 1 shows no hypersensitivity to nitric oxide

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Hansen JM, Thomsen LL, Olesen J & Ashina M. Familial hemiplegic migraine type 1 shows no hypersensitivity to nitric oxide. Cephalalgia 2008; 28:496–505. London. ISSN 0333-1024

Familial hemiplegic migraine type 1 (FHM-1) is a dominantly inherited subtype of migraine with aura and transient hemiplegia associated with mutations in the CACNA1A gene. FHM-1 shares many phenotypical similarities with common types of migraine, indicating common neurobiological pathways. Experimental studies have established that activation of the nitric oxide–cyclic guanosine monophosphate (NO–cGMP) pathway plays a crucial role in migraine pathophysiology. Therefore, we tested the hypothesis that CACNA1A mutations in patients with FHM-1 are associated with hypersensitivity to NO–cGMP pathway. We included eight FHM-1 patients with R583Q and C1369Y mutations and nine healthy controls, who received intravenous infusions of 0.5 µg kg⁻¹ min⁻¹ glyceryl trinitrate (GTN) over 20 min. We recorded: headache intensity on a verbal rating scale; mean flow velocity in the middle cerebral artery (VmeanMCA) by transcranial Doppler; diameter of the superficial temporal artery (STA) by Dermascan. One patient reported migraine without aura 5 h after start of the GTN infusion. No aura was reported. The AUCheadache in the immediate phase was more pronounced in patients than in controls (P = 0.01). In the 14 h following GTN infusion, there was no difference in the AUCheadache between patients and controls (P = 0.17). We found no difference in the AUCVmeanMCA (P = 0.12) or AUCSTA (P = 0.71) between FHM-1 patients and controls. None of the control persons reported migraine-like headache. FHM-1 patients do not show hypersensitivity of the NO–cGMP pathway, as characteristically seen in migraine patients with and without aura. This indicates that the pathophysiological pathways underlying migraine headache in FHM-1 may be different from the common types of migraine. © Blackwell Publishing Ltd Cephalalgia, 2008, 28, 496–505

Introduction

Familial hemiplegic migraine (FHM) is a rare, dominantly inherited subtype of migraine with aura (1). FHM is phenotypically characterized by fully reversible half-sided weakness and other aura symptoms preceding or accompanying a migrainous headache (2, 3). FHM type 1 (FHM-1) is caused by missense mutations in the CACNA1A gene on chromosome 19p13, encoding the α1A subunit of calcium channels (4), FHM type 2 (FHM-2) is caused by mutations in the ATP1A2 gene encoding the α2 subunit of a Na⁺, K⁺ ATPase (5, 6) and FHM type 3 (FHM-3) is caused by mutations in the SCN1A gene encoding a neuronal voltage gated sodium channel (7). FHM-1 and FHM 2 are caused
by several different mutations (8–16); in FHM-3, however, only two mutations have been described so far.

FHM-1 has many clinical similarities to migraine with (MA) and without aura (MoA) (17), and an epidemiological study of a population-based FHM cohort has shown that 65% of the FHM patients had MA and/or MoA (17, 18). However, it has been reported that MA and MoA are not associated with any of the known FHM mutations (19–22). Nevertheless, it could be expected that the clinical similarities between FHM, MA and MoA (17) would be caused by common neurobiological pathways underlying pathophysiological mechanisms.

The nitric oxide–cyclic guanosine monophosphate (NO–cGMP) pathway plays a fundamental role in migraine pathophysiology (23–25), and the glyceryl trinitrate (GTN) model of migraine has become established as a very robust and reproducible way of triggering typical attacks indistinguishable from the patient’s usual attacks (24, 26, 27). This hypersensitivity to NO might be a shared feature of both MoA/MA and FHM, and could be the basis of the frequent co-occurrence of these disorders.

The aim of the present study was to test the hypothesis that CACNA1A mutations in a genotyped group of FHM-1 patients are associated with hypersensitivity to NO, and that FHM-1 would thus share a disturbance of the NO–cGMP pathway with MA and MoA.

Methods

Eight FHM-1 patients with R583Q and C1369Y mutations [2M/6F, mean age 40 years (range 27–57 years)] (Table 1) and nine healthy controls [5M/4F, mean age 33 years (range 24–49 years)] were recruited. Inclusion criteria for the patients were a diagnosis of FHM-1, with a known mutation in the CACNA1A gene according to the criteria of the International Headache Society (IHS) (3). The patients were recruited from a Danish population-based sample of FHM patients earlier reported (28, 29). This cohort consisted of 20 FHM-1 patients with known mutations. All 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 eight out of 20 patients (40%) from the Danish population-based cohort. The most frequent CACNA1A mutation (T666M) (2, 30, 31)

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was not present in any of the participating patients, but R583Q, the second most frequent mutation (10, 32) and the most prevalent Danish FHM-1 mutation (29) was represented.

Exclusion criteria for the patients were: any daily medication apart from oral contraceptives; serious somatic or psychiatric diseases. The control healthy subjects did not have a history of migraine or any other type of headache (except episodic tension-type headache for < 1 month). The study was approved by the Ethics Committee of the County of Copenhagen (KA 04088) and was undertaken in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000. All subjects gave informed consent to participate. The study was registered at ClinicalTrials.gov (ãØåNCT00257985).

Experimental design

The study design was balanced and single-blinded. The laboratory technicians performing the measurements were blinded with respect to patients and controls. All subjects received a continuous intravenous infusion of 0.5 \( \mu \text{g} \text{kg}^{-1} \text{min}^{-1} \) GTN over 20 min. The subjects were informed that GTN might induce headache in some individuals, but the timing or the type of headache were not discussed.

All subjects reported to the laboratory at 08.00 h headache-free. The intake of coffee, tea, cocoa or other methylxanthine-containing foods or beverages was not allowed for the last 8 h before the start of the study, to avoid a possible effect on cerebral blood flow. 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. The participant then rested for 30 min before baseline measurements of blood pressure, heart rate and ECG were done and the infusion started, using a time- and volume-controlled infusion pump (Braun Perfusor, Melsungen, Germany). Headache intensity, middle cerebral artery mean blood flow velocity (V\(_{\text{meanMCA}}\)), superficial temporal artery diameter, end-tidal partial pressure of \( \text{CO}_2 \) (\( \text{P}_\text{etCO}_2 \)), adverse events and vital signs were recorded at \( T_{-10} \) and then every 10 min until 120 min after start of infusion. The subjects were discharged from the hospital after finishing the measurements and were asked to complete a headache diary every hour until 12 h after discharge. The diary included headache characteristics and accompanying symptoms according to the IHS (3), any rescue medication taken and adverse events. Subjects were allowed to take rescue medication of their own choice at any time.

Headache intensity

Headache intensity was recorded on a verbal rating scale (VRS) from 0 to 10 [0, no headache; 1, a very mild headache (including a feeling of pressing or throbbing); 5, moderate headache; 10, worst imaginable headache] (24).

Cerebral haemodynamics

The mean velocity of blood flow in the middle cerebral artery (V\(_{\text{meanMCA}}\)) was recorded bilaterally by transcranial Doppler (TCD) with hand-held 2-MHz probes (Multidop X; DWL, Sipplingen, Germany), as previously described (33). All recordings were done by the same skilled technician (L.E.). To correct the V\(_{\text{MCA}}\) measurements for significant changes in \( \text{P}_\text{etCO}_2 \), changes were recorded in \( \text{P}_\text{etCO}_2 \) simultaneously with the TCD measurements using an open mask that caused no respiratory resistance (ProPac Encore®; Welch Allyn Protocol, Beaverton, OR, USA) (33).

Diameter of the superficial temporal artery

The diameter of the frontal branch of the superficial temporal artery (STA) was measured by a high-resolution ultrasonography unit (Dermascan C; Cortex Technology, Hadsund, Denmark; 20 MHz, bandwidth 15 MHz) as previously described (34).

Vital signs

Heart rate and blood pressure were measured every 10 min using an auto-inflatable cuff (ProPac Encore®; Welch Allyn Protocol). ECG (Cardiofax V; Nihon-Kohden, Shinjuku-ku, Tokyo, Japan) was monitored on an LCD screen and recorded on paper every 10 min.

Data analysis and statistical methods

All values are presented as mean ± S.D., 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 to 14 h after the start of infusion (2–14 h). Baseline was defined as –10 min before the start of infusion of each dose.

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Calculation of sample size was based on the detection of a difference between the proportion of patients and controls reporting GTN-induced migraine attack during delayed phase (2–14 h), at 5% significance with 80% power. We assumed that GTN would induce a migraine headache in approximately 80% of FHM-1 patients, as reported previously in common types of migraine (24, 27) and migraine-like headache in <10% of healthy controls (35, 36). We estimated that eight subjects should be included in each group (37), but it was planned to increase the sample size, in case more patients could be recruited.

The area under the curve (AUC) was used as summary measure for analysing differences between the groups [headache score, \(V_{\text{meanMCA}}\), diameter of STA, mean blood pressure (MAP), heart rate (HR) and \(P_{\text{CO}_2}\)]. The AUC was calculated according to the trapezium rule (38).

The primary end-points were differences in incidence of migraine headache and AUC for headache score (AUCheadache 0–120 min and AUCheadache 2 h–14 h), \(V_{\text{meanMCA}}\) (AUCVmeanMCA), STA (AUCSTA) and \(P_{\text{CO}_2}\) (AUCPco2) between groups. The secondary end-points were differences in the AUC for heart rate (AUCheart rate) and MAP (AUCMAP) between groups during the immediate phase and differences between baseline and peak responses (\(V_{\text{meanMCA}}\), diameter of the STA and heart rate) within groups and between groups at time of peak response (headache, \(V_{\text{meanMCA}}\), diameter of the STA and heart rate).

Statistical analysis was performed using an unpaired, two-way \(t\)-test except headache scores, where data are presented as medians and quartiles and tested with the Mann–Whitney test. The incidence of migraine and other adverse events between the groups was compared with Fisher’s exact test.

All analyses were performed with SPSS for Windows 14.0 (SPSS Inc., Chicago, IL, USA). Five percent (\(P < 0.05\)) was chosen as the level of significance.

### Results

All 17 subjects completed the study, and all subjects were headache free at baseline. The \(V_{\text{meanMCA}}\) recordings showed no differences between the sides at baseline (\(P > 0.05\)). Therefore, \(V_{\text{meanMCA}}\) of the right and left side were grouped and the average of the two was calculated. There were no differences between the groups at baseline for any other variables (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>FHM-1</th>
<th>Controls</th>
<th>(P)</th>
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<tbody>
<tr>
<td>(V_{\text{meanMCA}})</td>
<td>69.8 (± 9.9)</td>
<td>74.8 (± 7.6)</td>
<td>0.12</td>
</tr>
<tr>
<td>STA</td>
<td>1.09 (± 0.18)</td>
<td>1.13 (± 0.32)</td>
<td>0.75</td>
</tr>
<tr>
<td>HR</td>
<td>73.1 (± 16.1)</td>
<td>60.1 (± 13.4)</td>
<td>0.11</td>
</tr>
<tr>
<td>MAP</td>
<td>82.3 (± 15.6)</td>
<td>78.6 (± 7.8)</td>
<td>0.56</td>
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</table>

Groups compared with an unpaired \(t\)-test.

In one control subject we were only able to find a reliable Doppler signal from the MCA on one side of the head. There was one missing value for the heart rate measurements in one control subject (50 min) and three \(P_{\text{CO}_2}\) recordings were missing from two subjects in the control group (–10, 0 and 50 min).

### Aura

The GTN infusion did not induce an aura in any of the patients.

### Headache

One patient (patient 4, Table 1), but no controls reported a delayed headache fulfilling the criteria for MoA according to the IHS criteria (3). This patient reported peak headache intensity (5 on the VRS) at 7 h after start of the infusion. The headache was described as a bifrontal and retrobulbar pressing pain, associated with aggravation during physical activity, mild nausea and photophobia. The patient reported that the headache was similar to the usual migraine headache.

This patient suffered from migraine with typical aura attacks in addition to hemiplegic attacks. The incidence of reported migraine was not different in the two groups, with 12.5% (one out of eight) in the patient group, and 0% (0 out of nine) in the control group [95% confidence interval (CI) –0.11, 0.36; \(P = 0.47\)].

During the immediate phase (0–120 min) seven patients and three control subjects reported headache (Table 3). The AUCheadache 0–120 min in the patient group, 50 (28.7, 57.5), was significantly greater than in the control group, 0 (0, 20; \(P = 0.008\)). The peak headache occurred at 20 min, and the median peak headache in the patient group, 1.5 (0.3, 2), was higher than in the control group, 0 (0, 1; \(P = 0.046\)) (Fig. 1).

During the delayed phase (2–14 h), five patients and two controls reported headache (Table 3), in all cases without any associated symptoms such as...
nausea, photophobia or phonophobia. We found no difference in the AUC headache 2 h−14 h between FHM-1 patients, 2 (0, 5.4) and controls, 0 (0, 0; \( P = 0.167 \)) (Fig. 2). The median headache was 0 for all time points in the 2−14 h period.

Middle cerebral artery mean blood flow velocity

There was no difference in the AUC_{V_{\text{meanMCA}}} between FHM-1 patients (7617 ± 1001) and controls (8281 ± 969) during the immediate phase (\( P = 0.115 \)) (Fig. 3). The mean peak decrease in \( V_{\text{meanMCA}} \) compared with baseline occurred at 20 min, and was −21.1 ± 9% in the patient group and −20.0 ± 10% in the control group. The mean difference between patients and controls at 20 min was −1.1% (95% CI −7.6, 5.4; \( P = 0.74 \)). There were no differences in the \( P_{\text{etCO}_2} \) recordings (0−120 min) during TCD scans between FHM patients and controls (\( P = 0.37 \)).

Superficial temporal artery

There was no difference in the AUC_{STA} between FHM-1 patients (159 ± 25) and controls (165 ± 39) during the immediate phase (\( P = 0.71 \)) (Fig. 4). The peak increase in the STA diameter compared with baseline occurred at 10 min, and was 45 ± 17% in the patient group and 52 ± 22% in the control group. The mean difference in response between patients and controls at 10 min was −7.1% (95% CI −27, 13; \( P = 0.46 \)).

Mean arterial blood pressure and heart rate

We found no difference in the AUC_{MAP} (\( P = 0.76 \)) or AUC_{HR} 0−120 min (\( P = 0.11 \)) between patients and controls.

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**Table 3** Number of patients and controls reporting headache and migraine headache

<table>
<thead>
<tr>
<th></th>
<th>FHM-1</th>
<th>Controls</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td>Immediate headache (0−120 min)</td>
<td>7</td>
<td>3</td>
<td>0.05</td>
</tr>
<tr>
<td>Delayed headache (2−14 h)</td>
<td>5</td>
<td>2</td>
<td>0.15</td>
</tr>
<tr>
<td>Migraine according to ICHD</td>
<td>1</td>
<td>0</td>
<td>0.47</td>
</tr>
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</table>

Groups compared with Fisher's exact test.

ICHD, International Classification of Headache Disorders.
controls during the immediate phase. There was a significant increase in HR between baseline and the peak response at 20 min in FHM patients \( (P = 0.011) \) and at 10 min in controls \( (P = 0.017) \).

**Adverse events**

During the immediate period \( (0–120 \text{ min}) \), two patients and 0 controls reported flushing \( (P = 0.20) \); one patient and five controls reported palpitations \( (P = 0.13) \); and one control reported heat sensation \( (P = 1.0) \).

**Discussion**

This is the first study using the GTN migraine provocation model to explore the functional consequences of the \( R583Q \) and \( C1369Y \) gene mutations in FHM-1 patients. The major finding of the present study was that activation of the NO–cGMP pathway failed to induce migraine aura or migraine headache in patients with FHM-1. This finding is in sharp contrast to results in migraine patients with and without aura, where GTN induces migraine in 50–80% of patients \( (26, 27, 36) \).

GTN failed to induce a migraine aura

Cortical spreading depression (CSD), discovered by Leão \( (39) \), has been linked to migraine aura pathogenesis in observational \( (40) \), animal \( (41) \) and human studies \( (42–44) \). Cerebral blood flow (CBF) studies during hemiplegic aura showed a spreading cortical hypoperfusion \( (45) \), similar to MA \( (46) \), which suggests that CSD is the most likely mechanism of hemiplegic aura \( (47, 48) \). It still remains unresolved, why migraine patients are more susceptible to CSD. In the case of FHM, animal studies of \( CACNA1A \) knock-in mice carrying the human \( R192Q \) mutation showed increased susceptibility to CSD \( (49) \). It has been proposed that \( CACNA1A \) mutations lead to increased release of glutamate, and thus increased susceptibility to CSD \( (50) \).

The molecular mechanisms of the initiation and propagation of CSD are not fully understood. However, animal studies have reported that CSD is associated with the release of NO \( (51, 52) \), and NO has also been linked to the modulation of the calcium entry through P/Q type calcium channels \( (53) \), and the transduction between neuronal activity and increased CBF after CSD \( (54) \). Furthermore, Read and colleagues have shown that GTN stimulates the release of NO in response to CSD \( (52) \) and that CSD increases the levels of cGMP in the cortex and brainstem \( (55) \). 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. \( (36) \) in a study attempting to trigger migraine aura in 12 patients with pure MA, i.e. without any coexisting MoA. The study showed that 50% of the patients developed migraine headache, but none developed migraine aura. In another study of 21 patients with
MA, i.v. GTN induced reproducible aura in one patient (27), and in a study of 22 patients, sublingually applied GTN induced aura in three patients (56).

Collectively, these data suggest that GTN may be able to induce aura in few migraine patients, i.e. with a relatively low rate of aura induction. The genotype of FHM-1 may be associated with a decreased CSD threshold, and it might therefore be expected that GTN would 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. Thus, our data suggest that the NO–cGMP pathway is not involved in the pathogenesis of migraine aura in FHM-1 patients with the R583Q/C1369Y mutations.

GTN failed to induce migraine headache

Experimental studies in migraineurs have demonstrated that the NO–cGMP pathway plays an important role in triggering (23–25, 27, 36, 57) and maintaining (58) migraine headache. Interestingly, the study by Christiansen et al. (36) has shown that although GTN failed to induce aura, 50% of MA patients developed migraine headache. This indicates that the NO–cGMP neurobiological pathway is involved in triggering migraine headache in patients with MA. Since FHM-1 and MA patients share clinical features such as non-hemiplegic aura symptoms, a similar headache phase and similar associated symptoms (17), we hypothesized that GTN infusion would induce a migraine headache in most FHM-1 patients. The present study has shown, however, that GTN failed to trigger more migraine headache in FHM-1 patients than in healthy controls, and the reported pain intensity during the delayed phase was not different between the groups. This is in sharp contrast to earlier findings, where exogenous NO has been found to cause more episodes of migraine headache in migraine patients than in controls (26, 27), and also to induce a more pronounced headache intensity response in migraine patients than in healthy control subjects (23, 36).

The present results therefore suggest that the R583Q/C1369Y mutations do not cause hypersensitivity to GTN and consequently seem to affect neurobiological pathways other than those in MA and MoA.

Two out of eight FHM-1 patients had both FHM-1 and MA and only one of these, with the R583Q mutation, developed delayed headache fulfilling the criteria for MoA. This is similar to the placebo rate of migraine induction in a study, where one out of 10 MoA patients developed a migraine attack after placebo (24). Interestingly, family members (n = 5) of this patient with the same mutation but without known coexisting common types of migraine did not develop migraine.

In MoA patients, the sensitivity to i.v. GTN seemed not to depend on the frequency of spontaneously occurring migraine attacks (59), whereas in a large study, applying GTN sublingually, increased sensitivity to NO has been linked to increased attack frequency in MoA patients (56). This study also examined MA patients, where no relationship was found between sensitivity to NO and frequency of attacks. Because FHM-1 is a subtype of MA and the FHM-1 patient experiencing the migraine attack after GTN suffered from MA, it seems that the difference in attack frequency is unlikely to affect our conclusion.

In the light of these surprising findings, one might suggest that neurobiological pathways responsible for migraine headache in coexisting MA are distinct from pathways responsible for migraine headache in FHM-1 patients with the R583Q/C1369Y mutations.

In line with previous studies on migraine patients (23), the FHM-1 patients developed more immediate headache (0–2 h) than controls. Arterial dilation may cause headache (60), and GTN infusion causes a more pronounced dilation of extra- and intra-cerebral arteries in migraine patients than in controls (61). In contrast, we found no differences in V_{meanMCA} 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 MoA patients (61). It also shows that the difference in immediate headache between FHM-1 and controls is unlikely to be caused by vasodilation.

Surprisingly few controls developed headache, compared with our earlier studies using the NO–cGMP model. The incidence of immediate headache in the control group, however, is similar to a large study by Sances et al. (56). Kinetic studies in the R593Q mutation (62) have shown that FHM mutated human CaV2.1 channels display an increased open probability, thus allowing FHM-1 channels to carry larger Ca^{2+} fluxes than in the wild type (63). Animal studies on knock-out rats for the Cav2.1 calcium channel have indicated that the P/Q-type calcium channels may have a pronociceptive role in inflammatory and neuropathic pain.
states (64). Based on these data, it could plausibly be suggested 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.

Methodological considerations

Epidemiological studies have shown that FHM is a very rare disease, with a prevalence of approximately 0.005% (28). We were able to recruit eight out of 20 patients (40%) from the Danish population-based cohort. We applied a single blinded design to avoid the risk of losing patients to follow-up in case of crossover design. Based on previous GTN studies and our hypothesis that FHM-1, MA and MoA share the hypersensitivity towards NO, our power calculation showed that eight subjects in each group would be enough to show statistical difference with respect to reported migraine attacks. The study, however, showed a non-significant difference in migraine induction between the two groups of only 12%. This difference is hardly clinically relevant, but we cannot exclude a small effect.

The present study suggests that FHM-1 patients do not show hypersensitivity of the NO–cGMP pathway, as characteristically seen in MoA and MA. Furthermore, the present data indicate that pathophysiological pathways underlying migraine headache in FHM-1 may be different from the common types of migraine (MA and MoA). Our material does not allow a separate evaluation of each mutation. Further studies are warranted to examine this, and explore whether FHM-2 and FHM-3 also differ from the common types of migraine.

Acknowledgements

The authors thank all participating FHM-1 patients, and laboratory technicians Kirsten Brunsgaard and Lene Elkjaer for their dedicated and excellent assistance. The authors wish to thank Associate Professor Lene Theil Skovgaard (Department of Biostatistics, University of Copenhagen) for statistical advice. The study was supported by the University of Copenhagen, the A.P. Møller Foundation for advancement of medical science, the Cool Sorption Foundation, The Danish Medical Association Research Fund, Danish Headache Society, Ms Else Torp and Flemming Jensen Foundation, Ms Lily Benthine Lund Foundation, Jacob Madsen and Olga Madsen Foundation, deCODE genetics, The Lundbeck Foundation, Breakthrough Breast Cancer, and the European Community [EUROHEAD (LSHM-CT-2004-504837)].

References


Read SJ, Hirst WD, Upton N, Parsons AA. Cortical spreading depression produces increased cGMP levels in cortex and brain stem that is inhibited by tonabersat (SB-220453) but not sumatriptan. Brain Res 2001; 891:69–77.


Luvisetto S, Marinelli S, Pansiti MS, D’Amato FR, Fletcher CF, Pavone F, Pietrobon D. Pain sensitivity in mice lacking the Ca(v)2.1alpha(1) subunit of P/Q-type Ca(2+) channels. Neuroscience 2006;142:823–32.
Familial hemiplegic migraine type 2 does not share hypersensitivity to nitric oxide with common types of migraine

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Familial hemiplegic migraine type 2 (FHM-2) and common types of migraine show phenotypic similarities which may indicate a common neurobiological background. The nitric oxide–cyclic guanosine monophosphate (NO–cGMP) pathway plays a crucial role in migraine pathophysiology. Therefore, we tested the hypothesis that ATP1A2 mutations in patients with FHM-2 are associated with hypersensitivity to NO–cGMP pathway. Eight FHM-2 patients with R202Q, R763C, V138A and L764P mutations and nine healthy controls received intravenous infusions of 0.5 μg kg⁻¹ min⁻¹ glyceryl trinitrate (GTN) over 20 min. We recorded the following variables: headache intensity on a verbal rating scale; mean flow velocity in the middle cerebral artery (VmeanMCA) by transcranial Doppler; diameter of the superficial temporal artery (STA) by ultrasound. The primary end-points were differences in incidence of migraine headache and area under the curve (AUC) for headache score during an immediate phase (0–120 min) and a delayed phase (2–14 h) after start of infusion. We found no difference in the incidence of reported migraine between FHM-2 patients, 25% (two out of eight), and controls, 0% (0 out of nine) (95% confidence interval −0.06, 0.56) (P = 0.21). The AUCheadache in the immediate (P = 0.37) and delayed (P = 0.09) phase was not different between patients and controls. The GTN infusion resulted in a biphasic response in patients. During the immediate phase, the median peak headache occurred at 30 min and tended to be higher in patients, 1 (0, 3.8), than in controls, 0 (0, 1) (P = 0.056). During the delayed phase, the median peak headache occurred 4 h after the start of the infusion and was significantly higher in patients, 2.5 (0, 3), than in controls, 0 (0, 0) (P = 0.046). We found no difference in the AUCVmeanMCA (P = 0.77) or AUCSTA (P = 0.53) between FHM-2 patients and controls. GTN infusion failed to induce more migraine in FHM-2 patients than in controls. The pathophysiological pathways underlying migraine headache in FHM-2 may be different from the common types of migraine.

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Introduction

Familial hemiplegic migraine type 2 (FHM-2) is a rare, dominantly inherited subtype of migraine with aura (MA) (1). FHM-2 is associated with mutations in the ATP1A2 gene encoding the a2 subunit of a Na+, K+ ATPase (2, 3), and > 20 different FHM-2 mutations have been identified; for a review see (4). The identification of the mutated FHM genes (5–7) has lead to the assumption that migraine may be a channelopathy, and stimulated research in the link between genotype and phenotype (8).

Migraine attacks in FHM-2 are characterized by fully reversible half-sided weakness preceding or accompanying a migrainous headache (9). Phenotypically, there are many clinical similarities between FHM-2 and MA and migraine without aura (MoA) (9). In a population-based study of genotyped FHM patients, > 60% of the FHM-2 patients had one or two other forms of migraine attacks (10). Although MA and MoA are not associated with any of the known FHM mutations (11–14), FHM and the common types of migraine may share common neurobiological pathways underlying the pathogenesis of migraine.

Activation of the nitric oxide–cyclic guanosine monophosphate (NO–cGMP) pathways plays a fundamental role in migraine pathophysiology (15), as shown by administration of glyceryl trinitrate (GTN), which has been shown to trigger migraine attacks indistinguishable from the usual attacks in migraine patients (16–18). Recently, we examined a group of genotyped FHM-1 patients using the GTN model of migraine (19). We found that the R583Q and C1369Y mutations of the CACNA1A gene were not associated with hypersensitivity of the NO–cGMP pathway. Whether this pathway is a likely mechanism of FHM-2 remains unknown. 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.

In the present study, we tested the hypothesis that ATP1A2 mutations in a genotyped group of FHM-2 patients might be associated with hypersensitivity to the NO–cGMP pathway.

Design and methods

Eight FHM-2 patients with R202Q, R763C, V138A and L764P mutations [5 M/3 F, mean age 45 years (range 19–59 years)] (Table 1) and nine healthy

Table 1 Clinical characteristics of eight FHM-2 patients with R202Q, R763C, V138A and L764P mutations

<table>
<thead>
<tr>
<th>Subj.</th>
<th>Mutation Ataxia</th>
<th>Headache accompanying hemiplegic attacks</th>
<th>Comorbidity</th>
<th>Migraine with aura</th>
<th>Migraine without aura</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R202Q</td>
<td>&lt;1/year 15–30 min</td>
<td>Throbbing/6-7</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
<tr>
<td>2</td>
<td>R202Q</td>
<td>3–6 h</td>
<td>Press/6</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
<tr>
<td>3</td>
<td>R202Q</td>
<td>3–4/year</td>
<td>Press/6</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
<tr>
<td>4</td>
<td>R202Q</td>
<td>1/year 15–30 min</td>
<td>Throbbing/6-7</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
<tr>
<td>5</td>
<td>R763C</td>
<td>1/year 15–30 min</td>
<td>Throbbing/6-7</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
<tr>
<td>6</td>
<td>V138A</td>
<td>1/year 15–30 min</td>
<td>Throbbing/6-7</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
<tr>
<td>7</td>
<td>L764P</td>
<td>1/year 15–30 min</td>
<td>Throbbing/6-7</td>
<td>No/yes/yes/yes/yes</td>
<td>No/yes/yes/yes/yes</td>
</tr>
</tbody>
</table>

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volunteers [5 M/4 F, mean age 33 years (range 24–49 years)] were recruited. The study was part of the EUROHEAD project (http://www.eurohead.org), and patients were recruited and examined in both Denmark and Italy, using the same methods. The measurements in Italy were conducted by an experienced member of the Danish research group (H.S.), to reduce interobserver variability.

We recruited seven of 13 patients (55%) with known ATP1A2 mutations from The Danish population-based cohort (10) and one patient from the Department of Neurology, Misericordia Hospital, Grosseto and University of Milan, Italy.

Inclusion criteria for the patients were a diagnosis of FHM-2 according to the criteria of the International Headache Society (IHS) (1), with a known mutation in the ATP1A2 gene. Exclusion criteria for the patients were: any daily medication apart from oral contraceptives; serious somatic or psychiatric diseases. The control healthy subjects did not have a history of migraine or any other type of headache (except episodic tension-type headache less than once a month). None of the healthy controls had a family history of migraine.

The study was approved by the Ethics Committees of the County of Copenhagen (KA 04088) and County of Grosseto (731/CESF) and was undertaken in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000. All subjects gave informed consent to participate in the study.

Experimental design

The study design was non-randomized, controlled and single-blinded. The laboratory technicians performing the measurements in Denmark were blinded with respect to the subject status as patient or control. All subjects received a continuous intravenous infusion of 0.5 μg kg⁻¹ min⁻¹ GTN over 20 min. All subjects were informed that GTN might induce headache in some individuals, but the timing and type of headache were not discussed.

All subjects reported headache-free to the laboratory. The intake of coffee, tea, cocoa or other methylxanthine-containing foods or beverages was not allowed for the last 8 h before the start of the study, to avoid a possible effect on the cerebral blood flow. 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. The participant then rested for 30 min before baseline measurements of blood pressure, heart rate and electrocardiogram were done and the infusion started, using a time- and volume-controlled infusion pump (Braun Perfusor, Melsungen, Germany). Headache intensity, mean blood flow velocity of the middle cerebral artery (VmeanMCA), diameter of the superficial temporal artery, end-tidal partial pressure of CO₂ (Pco₂), adverse events and vital signs were recorded at T10, and then every 10 min until 120 min after start of infusion. The subjects were discharged from the hospital after finishing the measurements and were asked to complete a headache diary every hour until 12 h after the discharge. The diary included headache characteristics and accompanying symptoms according to the IHS (1), any rescue medication taken and adverse events. Subjects were allowed to take rescue medication of their own choice at any time.

Headache intensity

Headache intensity was recorded on a verbal rating scale (VRS) from 0 to 10 (0, no headache; 1, a very mild headache (including a feeling of pressing or throbbing); 5, moderate headache; 10, worst imaginable headache) (20).

Cerebral haemodynamics

The mean velocity of blood flow in the middle cerebral artery (VmeanMCA) was recorded bilaterally by transcranial Doppler (TCD) with hand-held 2-MHz probes (Multidop X; DWL, Sipplingen, Germany), as previously described (21). To correct VmeanMCA measurements for possible significant changes in End-tidal PCO₂ (Pco₂), we recorded changes in Pco₂ simultaneously with the TCD measurements using an open mask that caused no respiratory resistance (ProPac Encore®; Welch Allyn Protocol, Beaverton, OR, USA).

Diameter of the superficial temporal artery

The diameter of the frontal branch of the superficial temporal artery (STA) was measured by a high-resolution ultrasonography unit (Dermascan C; Cortex Technology, Hadsund, Denmark: 20 MHz, bandwidth 15 MHz), as previously described (22).

Vital signs

Heart rate and blood pressure were measured every 10 min using an auto-inflatable cuff (ProPac Encore®; Welch Allyn Protocol). ECG (Cardiofax V,
Nihon-Kohden; Shinjuku-ku, Tokyo, Japan) was monitored on an LCD screen and recorded on paper every 10 min.

Data analysis and statistical methods

All values are presented as mean ± S.D., 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 (2–14 h). Baseline was defined as –10 min before the start of infusion.

Sample size calculation was based on the detection of a difference in proportion of patients and controls reporting GTN-induced migraine attack during the delayed phase (2–14 h), at 5% significance with 80% power. We assumed that GTN would induce a migraine headache in approximately 80% of FHM-2 patients, as reported previously in MA and migraine-like headache in <10% of healthy controls (23). We therefore estimated that eight subjects should be included in each group (24).

The area under the curve (AUC) was used as summary measure for analysing differences between the groups [headache score, $V_{\text{meanMCA}}$, diameter of STA, mean blood pressure (MAP), heart rate (HR) and $P_{\text{etCO}_2}$]. The AUC was calculated according to the trapezium rule (25). The primary end-points were differences in incidence of migraine headache and in the AUC for headache score ($AUC_{\text{headache } 0\text{–120 min}}$ and $AUC_{\text{headache } 2\text{h–14h}}$), $V_{\text{meanMCA}}$ ($AUC_{\text{VmeanMCA}}$), STA ($AUC_{\text{STA}}$) and $P_{\text{etCO}_2}$ ($AUC_{\text{PetCO}_2}$) between groups. The secondary end-points were differences in the AUC for heart rate ($AUC_{\text{heart rate}}$) and MAP ($AUC_{\text{MAP}}$) between groups during the immediate phase and differences between baseline and peak responses ($V_{\text{meanMCA}}$, diameter of the STA and heart rate) within groups and between groups at time of peak response (headache, $V_{\text{meanMCA}}$, diameter of the STA and heart rate).

Statistical analysis was performed using an unpaired, two-way $t$-test except for headache scores, where data are presented as medians and quartiles and tested with Mann–Whitney test. The prevalence of migraine and other adverse events between the groups was compared with Fisher’s exact test. All analyses were performed with SPSS for Windows 14.0 (Chicago, IL, USA). Five per cent ($P < 0.05$) was chosen as the level of significance.

Results

All 17 subjects completed the study, and all subjects were headache-free at baseline (–10 min). The $V_{\text{meanMCA}}$ recordings showed no differences between the sides at baseline ($P > 0.05$) and therefore the average of the two sides was used in the statistical calculations.

There was no difference between the groups at baseline for any other variables (Table 2).

In one control subject we could find a reliable Doppler signal only from the MCA on one side of the head, which was then used in the calculations. There were 16 missing Doppler recordings in the patient group ($n = 5$) and two in the control group ($n = 2$), because of difficulties localizing the correct vessel. There were four missing recordings of the STA in one patient, mainly because of lack of time as this patient was examined in Italy without the help of the usual laboratory technicians. There was one missing value for the heart rate in the control group (50 min), and the CO2 recordings were not done in two patients because the mask caused discomfort.

Migraine attacks

No difference was found in the incidence of reported migraine between FHM-2 patients, 25% (2/8), and controls, 0% (0/9) [95% confidence interval (CI) −0.06, 0.56] ($P = 0.21$).

Table 2 Baseline values (mean ± S.D.)

<table>
<thead>
<tr>
<th></th>
<th>FHM-2</th>
<th>Controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean blood flow velocity of the middle cerebral artery</td>
<td>82.2 (± 23.5)</td>
<td>74.8 (± 7.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Superficial temporal artery diameter</td>
<td>1.22 (± 0.25)</td>
<td>1.13 (± 0.32)</td>
<td>0.51</td>
</tr>
<tr>
<td>Heart rate</td>
<td>73.0 (± 13.7)</td>
<td>61.6 (± 14.2)</td>
<td>0.187</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
<td>88.8 (± 13.3)</td>
<td>78.6 (± 7.8)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Groups compared with an unpaired $t$-test.

FHM-2, familial hemiplegic migraine type 2.
Patient 6 (Table 1) reported peak headache intensity in the immediate phase 20 min after start of the GTN infusion (4 on the VRS) and a peak headache intensity in the delayed phase 4 h after start of the infusion (3 on the VRS). The patient was not pain-free at any time after the infusion. The headache was described as a bilateral, throbbing pain, associated with aggravation by physical activity, mild nausea, phonophobia and photophobia, and thus fulfilled the IHS criteria during both the immediate and the delayed phase (1). No aura was reported by this patient.

Patient 7 (Table 1) reported to the laboratory headache-free and scored 0 on the VRS at baseline (−10 min). However, just before the start of the infusion (0 min), the patient reported a mild sensation of pressure in the head, but no headache (1 on the VRS). This patient reported peak headache intensity in the immediate phase 30 min after start of the GTN infusion (7 on the VRS), and a peak headache intensity in the delayed phase 12–14 h after start of the infusion (10 on the VRS). The patient was not pain-free at any time after the infusion. The headache was described as a bilateral, constant pain, but without any associated features in the immediate phase. During the delayed phase (7.2 h after start of infusion), the patient experienced a visual aura with fortification spectra (duration of 30 min), followed by hemiplegia of the left arm and leg (with a duration of approximately 20 min, starting in the arm and then the leg). Before and during the aura, the patient had bilateral constant headache associated with aggravation through physical activity, mild nausea, phonophobia and photophobia, and thus fulfilled the IHS criteria for hemiplegic migraine, apart from the fact that the headache was present before the onset of hemiplegic aura symptoms (1). The patient took 50 mg diclofenac and went to sleep for 3.5 h. The aura symptoms had vanished as the patient woke up again, but the headache had worsened (VRS 10), and the patient took Ibuprofen, 600 mg. The patient reported that the symptoms of the hemiplegic attack were similar to her usual attacks.

Non-migraine headache

During the immediate phase (0–120 min), four patients and three control subjects reported headache (Table 3). There was no difference in the AUC_{headache} 0–120 min between patients, 10 (0, 384) and controls, 0 (0, 20) (P = 0.37). The median peak headache occurred at 30 min and tended to be higher in patients, 1 (0, 3.8), than in controls, 0 (0, 1) (P = 0.056) (Fig. 1).

During the delayed phase (2–14 h), five patients and two controls reported headache (Table 3). No difference was found in the AUC_{headache} 2h–14h between FHM-2 patients, 6.5 (0, 12.3), and controls, 0 (0, 0) (P = 0.09) (Fig. 1). Median peak headache occurred 4 h after the start of the infusion and was significantly higher in patients, 2.5 (0, 3), than in controls, 0 (0, 0) (P = 0.046) (Fig. 1).

Middle cerebral artery mean blood flow velocity

There was no difference in the AUC_{VmeanMCA} between FHM-2 patients and controls during the immediate phase (P = 0.77) (Fig. 2). The mean peak change in V_{meanMCA} compared with baseline occurred at 20 min, and was −22.0 ± 6.9% in the patient group and −20.0 ± 9.8% in the control group. The mean difference between patients and controls at 20 min was
There was no difference in the AUC$_{\text{STA}}$ between FHM-2 patients and controls during the immediate phase ($P = 0.53$) (Fig. 3). The peak change in the STA diameter compared with baseline occurred at 20 min and was $32 \pm 11\%$ in the patient group and $52 \pm 22\%$ in the control group. The mean difference in response between patients and controls at 20 min was $-19.5\%$ (95% CI $-37.4$, $-1.64$) ($P = 0.035$).

**Superficial temporal artery**

There was no difference in the AUC$_{\text{STA}}$ between FHM-2 patients and controls during the immediate phase ($P = 0.53$) (Fig. 3). The peak change in the STA diameter compared with baseline occurred at 20 min and was $32 \pm 11\%$ in the patient group and $52 \pm 22\%$ in the control group. The mean difference in response between patients and controls at 20 min was $-19.5\%$ (95% CI $-37.4$, $-1.64$) ($P = 0.035$).

**Measurable arterial blood pressure and heart rate**

We found no difference in the AUC$_{\text{MAP \ 0-120 min}}$ ($P = 0.35$) or AUC$_{\text{HR \ 0-120 min}}$ ($P = 0.11$) between patients and controls during the immediate phase (Fig. 4). There was a significant difference in heart rate between baseline and the peak response at 10 min in both FHM patients ($P = 0.044$) and controls ($P = 0.025$). The mean increase in heart rate (HR) at 10 min was $9.5 \pm 12.3\%$ in the patient group and $15.5 \pm 16.8\%$ in the control group ($P = 0.45$).

**Adverse events**

During the immediate period (0–120 min), one patient and no controls reported flushing ($P = 0.47$); two patients and five controls reported palpitations ($P = 0.33$); and four patients and one control reported heat sensation ($P = 0.13$) (Fisher’s exact test).

**Discussion**

This is the first study on FHM-2 patients using the GTN migraine provocation model to explore the functional consequences of the R202Q, R763C, V138A and L764P gene mutations in FHM-2
patients. The major finding of the present study is that activation of the NO-cGMP pathway failed to induce more migraine aura or migraine headache in FHM-2 patients than in healthy volunteers. This finding is in sharp contrast to previous findings in patients with common types of migraine. The question is how to reconcile this apparent reduced sensitivity to a known migraine trigger with the FHM-2 genotype?

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 (3). Reduced activity of the Na+, K+-ATPase may reduce the gradients required to drive the astrocytic glutamate transporter (26), and haploinsufficiency of the α2 Na+, K+-ATPase may thus reduce the removal of glutamate and lower the threshold for cortical spreading depression (CSD) (27). Pharmacological inactivation of the Na+ K+-ATPase (similar to the effects of FHM-2 mutations) causes CSD-like depolarization (28), and stimulation of the NO-cGMP pathway has been shown to inhibit Na+K+-ATPase activity (29, 30). Experiments in rats have shown that CSD in both the cortex (31) and hippocampus (32) may activate the trigeminovascular system, thereby linking migraine aura and the triggering of migraine headache (33). Collectively, these data indicate that FHM-2 patients might show a reduced threshold for CSD and, thus, induction of aura and increased susceptibility to migraine headache.

The present data have shown, however, that GTN failed to induce more migraine aura in the FHM-2 patients than in healthy controls. This result corresponds to findings in patients with migraine with typical aura, where GTN rarely (18) or never (23) induces aura, and is in concordance with recent data from FHM-1 patients (19).

It is well established that MA and MoA patients share a common hypersensitivity to activation of the NO-cGMP pathway, because infusion of GTN causes migraine or migraine-like headache in about 50% of MA patients and up to 80% of MoA patients (15, 17, 18, 34). We would therefore expect the headache response after GTN in FHM-2 to be similar to these figures, because spontaneous migraine headache in FHM is similar to MA and MoA (1, 9).

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

Six patients had migraine comorbidity of MoA (n = 2) and MA (n = 4). 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 coexisting MA or MoA vs. ‘pure’ FHM-2 patients. Such a study would be highly relevant, but difficult to set up due to the rarity of these patients.

Based on the present results, we can not exclude a small hypersensitivity to NO in FHM-2 patients with co-occurring MoA and MA, but this effect is probably too small to be clinically relevant.

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. 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 (35), we can not rule out that FHM-2 patients may be more sensitive to the GTN provocation than healthy volunteers.

In the present study, one patient reported hemiplegic aura and migraine headache after GTN. This patient came twice to the laboratory for the experiments. At the first visit she was excluded because of tension-type like headache before the experiment. During the second visit she reported headache-free to the laboratory but complained of a pressing sensation in the head (1 on the VRS), just before infusion. This patient reported that she was going through a difficult period of her life (changing job) and was quite stressed. We can therefore not entirely rule out the possibility that the reported attack was in fact a spontaneous one. Another possibility is that this particular mutation is associated with hypersensitivity of the NO-cGMP pathway. To clarify whether the hemiplegic aura could be triggered again, we could have repeated the GTN infusion as previously described in migraine with typical aura (18), but decided against it because the strain of inducing a hemiplegic attack is considerable. The two patients reporting migraine (as well as a third patient not reporting migraine) took non-steroidal anti-inflammatory drugs as rescue medication for the headache. This treatment might affect the results by reducing the headache (36), but is unlikely to affect our results, as the majority of our patients did not need rescue medication.

The FHM-2 patients had very infrequent hemiplegic attacks (Table 1), but a large study,
applying GTN sublingually, found no relationship between sensitivity to NO and frequency of MA attacks (34). FHM-2 is a subtype of MA (1), and the low attack incidence is therefore unlikely to have affected our results.

In summary, FHM-2 patients do not develop migraine attacks after GTN, but we can not rule out that FHM-2 patients are slightly more sensitive to GTN than healthy volunteers. Pathophysiological pathways underlying migraine headache in FHM-2 patients may thus be different from the pathways in patients with the common types of migraine.

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References

19 Hansen JM, Thomsen J, Olesen M. Ashina, familial hemiplegic migraine type 1 shows no hypersensitivity to nitric oxide. Cephalalgia; in press.
26 Abe K, Saito H. Involvement of Na\textsuperscript{+}–K\textsuperscript{+} pump in 1-glutamate clearance by cultured rat cortical astrocytes. Biol Pharm Bull 2000; 23:1051–4.
28 Balestrino M, Young J, Aitken P. Block of (Na\textsuperscript{+},K\textsuperscript{+}) ATPase with ouabain induces spreading depression-like depolarization in hippocampal slices. Brain Res 1999; 838:37–44.
30 Sato T, Kamata Y, Irifune M, Nishikawa T. Inhibition of purified (Na\textsuperscript{+},K\textsuperscript{+})-ATPase activity from porcine cerebral cortex by NO generating drugs. Brain Res 1995; 704:117–20.
Calcitonin gene–related peptide does not cause the familial hemiplegic migraine phenotype

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APPENDICE: PAPER 3

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migraine-like headache in migraine patients. CGRP receptor antagonism has documented efficacy in the treatment of migraine attacks. Recently, an orally available CGRP antagonist has shown efficacy in a phase 2 trial.

The mechanisms underlying the migraine-inducing effects of CGRP are 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 triggers such as CGRP is a logical step in a bottom-up approach to understanding the FHM phenotype.

We hypothesized that CACNA1A and ATP1A2 mutations in a group of genotyped FHM patients would be associated with hypersensitivity to GGRP, similar to that observed in MO patients. If that could be confirmed, it would indicate shared migraine mechanisms in FHM and MO and a possible role for CGRP antagonists in the treatment of FHM patients.

METHODS We recruited 9 FHM patients from a Danish population-based FHM cohort (7 FHM1 patients with the R583Q [n = 5] and C1369Y [n = 2] mutations [2 men and 5 women, mean age 39 years, range 29–56 years] and 2 FHM2 patients with the R202Q and R763C mutations [0 men and 2 women, mean age 39 years, range 29–56 years]) (Table 1) and 10 healthy volunteers (6 men and 4 women, mean age 32 years, range 23–42 years).

Inclusion criteria for the patients were a diagnosis of FHM with a known mutation in the CACNA1A and ATP1A2 genes according to the criteria of the International Headache Society (IHS). Exclusion criteria were any daily medication and serious somatic or psychiatric diseases. The healthy control subjects did not have a personal or a family history of migraine or any other type of headache. The study was approved by the ethics committees of the County of Copenhagen (KA 04088) and undertaken in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000. All subjects gave informed consent to participate in the study. The study was registered at ClinicalTrials.gov (NCT00358839).

Experimental design. The study design was balanced, controlled, and single-blinded. All measurements were performed by skilled technicians who were blinded in respect to patients and controls. All subjects received a continuous IV infusion of 1.5 μg/min CGRP over 20 minutes.

Human αGGRP was purchased from Clinalfa AG (Laulifingen, Switzerland). The subjects were informed that CGRP might induce headache in some individuals, but the timing or the type of headache was not discussed. Human γGGRP was infused into the superficial temporal artery.

All subjects reported to the laboratory at 08:00 AM headache-free. All procedures were performed in a quiet room with the subjects in the supine position. A venous catheter (Venflon®) was inserted into an antecubital vein, and after 30 minutes of rest, the infusion was started with an infusion pump (Braun Perfusor, Melsungen, Germany). Headache intensity, recorded on a verbal rating scale (VRS) from 0 to 10, was measured. Prior to the start of the infusion, the subjects were asked to complete a headache diary every hour until 14 hours after the start of the infusion. The diary included headache characteristics and accompanying symptoms according to the IHS. Subjects were allowed to take rescue medication of their own choice at any time.

Cerebral hemodynamics. The mean velocity of blood flow in the middle cerebral artery (VmeanMCA) was recorded bilaterally by transcranial Doppler with handheld 2-MHz probes (Multi dop X, DWL, Sipplingen, Germany), as previously described.

Diameter of the superficial temporal artery. The diameter of the frontal branch of the STA was measured by a high-resolution ultrasonography unit (Dermascan C, Cortex Imaging, Germany).

Table 1 Clinical characteristics of nine FHM patients with the R583Q, C1369Y, R202Q, and R763C mutations

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Mutation</th>
<th>Hemiplegic attacks</th>
<th>Headache accompanying hemiplegic attacks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency Duration</td>
<td>Duration</td>
</tr>
<tr>
<td>1</td>
<td>R763C</td>
<td>&lt;1/y 2–3 h</td>
<td>12–24 h</td>
</tr>
<tr>
<td>2</td>
<td>R583Q</td>
<td>&lt;1/y 1 h</td>
<td>3–4 h</td>
</tr>
<tr>
<td>3</td>
<td>R583Q</td>
<td>6–8/y 1 h</td>
<td>6–36 h</td>
</tr>
<tr>
<td>4</td>
<td>R583Q</td>
<td>3/y 45 min</td>
<td>6–24 h</td>
</tr>
<tr>
<td>5</td>
<td>R202Q</td>
<td>&lt;1/y 1.5–30min</td>
<td>12–24 h</td>
</tr>
<tr>
<td>6</td>
<td>C1369Y</td>
<td>5–10/y 1–2 h</td>
<td>6–24 h</td>
</tr>
<tr>
<td>7</td>
<td>C1369Y</td>
<td>&lt;1/y 6–24 h</td>
<td>4–5 h</td>
</tr>
<tr>
<td>8</td>
<td>R583Q</td>
<td>&lt;1/y 1–5 h</td>
<td>12–48 h</td>
</tr>
<tr>
<td>9</td>
<td>R583Q</td>
<td>10/y 1.0 min</td>
<td>1–2 days</td>
</tr>
</tbody>
</table>

FHM = familial hemiplegic migraine; MA = migraine with aura; MO = migraine without aura.
for MO according to the IHS criteria. During the immediate and a delayed headache fulfilling the criteria for MO among participants.

VmeanMCA showed no differences between right and left at baseline (p = 0.31 to 0.55; p = 0.58).

One patient (patient 1, table 1) reported an immediate and a delayed headache fulfilling the criteria for MO according to the IHS criteria. During the immediate phase, the peak headache was 3 on the VRS (20 minutes after the start of the infusion) and associated with photophobia, phonophobia, and aggravation by cough. The headache was bilateral and throbbing.

During the delayed phase, the patient reported bifrontal and throbbing headache with a peak intensity of 2, associated with mild photophobia, 9 h after the start of the infusion. There was a pain-free interval between the immediate and the delayed headache, and the headache was similar to her usual migraine headache. This patient had MO in addition to FHM2.

Another patient (patient 9, table 1) reported a delayed headache fulfilling the criteria for MO according to the IHS criteria. The headache started 3 h after the start of the infusion and had a peak intensity of 3 on the VRS. The headache was described as a left-sided and pressing headache, associated with mild nausea. This patient had pure hemiplegic migraine, and the headache was not similar to her usual migraine headache.

One patient (patient 4, table 1) reported a migraine-like delayed headache not fulfilling the IHS criteria for MO. This patient reported peak headache intensity of 5, with onset at 3 h and ending 7 h after the start of the infusion. There was no pain-free interval between the immediate and the delayed headache. The headache was bifrontal, pressing, and not aggravated by physical activity, associated only with mild nausea. This patient had pure hemiplegic migraine, and the headache was not similar to her usual migraine headache during FHM attacks.

In the control group, one subject reported a migraine-like headache during the delayed phase. The headache had a maximum intensity of 2, starting 2 h after the start of the infusion. The headache was described as bifrontal and pressing, associated with mild photophobia, phonophobia, and aggravation during physical activity. The subject had no family or previous history of migraine.

RESULTS All 19 subjects completed the study and were headache-free at baseline (−10 minutes). The VmeanMCA showed no differences between right and left at baseline (p > 0.05), and we therefore used the average of the two. There were no baseline differences in vascular variables between the groups (p > 0.05).

Aura. CGRP infusion did not induce aura in any of the participants.

Migraine headache. The incidence of reported migraine and migraine-like headache was not different in the two groups, with 22% (2 of 9) reporting migraine in the patient group, and 10% (1 of 10) reporting migraine-like headache in the control group (95% CI −0.31 to 0.55; p = 0.58).

One patient (patient 1, table 1) reported an immediate and a delayed headache fulfilling the criteria for MO according to the IHS criteria. During the immediate phase, the peak headache was 3 on the
Middle cerebral artery mean blood flow velocity. There was no difference in the AUC$_{V_{\text{meanMCA}}}$ between FHM patients and controls ($p = 0.73$; figure 2). The $V_{\text{meanMCA}}$ decreased between baseline and the peak response in both FHM patients ($p = 0.001$) and controls ($p < 0.001$). The peak decrease in $V_{\text{meanMCA}}$ was 9.6 ± 9.0% in the FHM group (30 minutes) and 9.6 ± 5.5% in the control group (20 minutes). The mean difference in peak response between patients and controls was $-0.05\%$ (95% CI $-7.5\%$ to $7.5\%$; $p = 0.98$).

Superficial temporal artery. The AUC$_{\text{STA}}$ was larger in the control group than in the FHM group ($p = 0.035$; figure 3). The STA increased between baseline and peak response in both FHM patients ($p < 0.001$) and controls ($p < 0.001$). The peak increase in STA was 42.8 ± 12.5% in the FHM group (25 minutes) and 42.5 ± 17.9% in the control group (20 minutes). The mean difference in peak response between patients and controls was $0.3\%$ (95% CI $-14.9\%$ to $15.5\%$; $p = 0.97$).

Mean arterial blood pressure and heart rate. The AUC$_{\text{HR 0–120 min}}$ was higher in the FHM group than in the control group ($p = 0.009$). There was a significant increase in HR between baseline and the peak response at 20 minutes in both FHM patients ($p < 0.001$) and controls ($p < 0.001$). We found no difference in the AUC$_{\text{MAP}}$ ($p = 0.82$) between FHM patients and controls during the immediate phase.

**DISCUSSION** The main outcome of the study was that infusion of the known migraine trigger CGRP did not induce more auras or more migraine-like headache in a group of genotyped FHM patients than in healthy controls.

The identification of the mutated FHM genes$^{1-3}$ stimulated interest in the link between genotype and phenotype using both molecular studies and animal models. Interestingly, a CACNA1A knock-in mouse showed increased susceptibility to cortical spreading depression (CSD).$^{19}$ Knock-in mice with the FHM2 and FHM3 mutations have not yet been developed. The functional consequences of the FHM mutations in humans, however, are not fully clarified, and the potential species differences should be considered. We hypothesized that genotyped FHM patients may be a suitable genetic migraine model. In particular, exposing these patients to known migraine triggers would help to elucidate the functional consequences of FHM mutations and would offer the opportunity to study the interplay between genotype and phenotype. Recently, we have examined genotyped FHM1 and FHM2 patients using the glyceryl trinitrate (GTN) model of migraine.$^{20,21}$ In contrast to our hypothesis, these mutations were not associated with hypersensitivity to activation of the nitric oxide–cyclic 3’,5’-guanosine monophosphate pathway. There might, however, be other pathways of importance for the FHM phenotype, such as the CGRP–cyclic adenosine 3’,5’-monophosphate pathway with its known importance in MO patients.$^9$

Migraine aura is likely to be the symptom of CSD,$^{22}$ and a model has been proposed that links FHM mutations with a propensity to CSD.$^{23}$ Animal studies have established a link between FHM1 mutations and increased susceptibility to CSD,$^{19}$ 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 because infusion of the neuropeptide endothelin 1 is able to cause CSD.$^{24}$

---

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

<table>
<thead>
<tr>
<th></th>
<th>FHM patients</th>
<th>Controls</th>
<th>p Value</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, 2-14 h</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 exact test.

FHM = familial hemiplegic migraine; ICHD = International Classification of Headache Disorders.

---

**Figure 1** Headache scores on a verbal rating scale during immediate and delayed phases after the start of CGRP infusion in 9 patients with FHM and 10 controls

Five familial hemiplegic migraine (FHM) patients and 7 control subjects reported headache in the immediate phase (0–120 minutes). The AUC$_{\text{headache 0-120 min}}$ did not differ between patients and controls ($p = 0.661$). During the delayed phase (2–14 hours), 3 patients and 5 controls reported headache. We found no difference in the AUC$_{\text{headache 2-14 h}}$ between patients and controls ($p = 1.00$). Thick lines are median pain scores. CGRP = calcitonin gene-related peptide; VRS = verbal rating scale.
ably via stimulation of phospholipase C. CGRP acts in part via the same mechanism.

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 important for pain signals in neurogenic inflammation, which has been linked to migraine pathogenesis. Sensitivity to CGRP may be a determinant of the nociceptive threshold because nociceptor function depends on the sensitivity to CGRP.

Patients with MO are hypersensitive to CGRP because infusion of CGRP causes migraine or migraine-like headache in these patients. A robust migraine response in FHM patients after CGRP would therefore indicate a common migraine mechanism in FHM and MO.

We found no difference in the prevalence of migraine attacks fulfilling the IHS criteria for MA or MO between FHM patients and controls. CGRP in a slightly larger dose (2 μg/min) induced delayed headache in 9 of 9 MO patients against only 3 of 9 patients in our study, but also severe hypotension in 2 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.

We found marked vascular effects of CGRP but no differences in the vascular variables between FHM and controls. Animal studies have demonstrated that CGRP-induced vasodilation is insufficient to activate nociceptors, but we know from other studies that migraine patients have an arterial hypersensitivity to other migraine triggers. This seems not to be the case for FHM patients, a finding corroborated by the larger AUCSTA in the control group. Even though the AUCSTA was larger in control group than in the FHM group (figure 3), the baseline measurements showed no difference between the groups. In our previous FHM studies using the GTN model of migraine, we found no difference in baseline and AUCSTA between controls and FHM patients.

Therefore, we suggest that it is unlikely that FHM patients share the hypersensitivity to CGRP with MO.

The phenotypical similarities and the great clinical overlap between FHM and common types of migraine 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 adenosine 3',5'-monophosphate pathways did not induce more migraine aura or migraine headache in FHM patients 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.

The new CGRP antagonists have no vascular effects that could limit their use in FHM patients, but based on our results, it seems unlikely that these compounds would be effective in the treatment of FHM patients.

We are aware that the present data should be interpreted with some caution. First, the present study was conducted in a limited number of subjects and without a placebo arm. Given that FHM is a very rare disease with a prevalence of 0.006%, and given the even smaller number of well-defined mutation carriers, we decided to apply a one-way active control design to minimize the risk of losing patients to follow-up in case of crossover design.

Second, CGRP-induced headache is not specific for migraineurs because CGRP may induce neuro-
vascular headache in healthy subjects. However, we know from experimental human model studies that many vasoactive substances induce headache in healthy subjects. Nevertheless, these models, including the CGRP model, 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 a reduced CGRP sensitivity in FHM patients.

Third, it could be argued that it is more relevant to compare migraine trigger hypersensitivity between FHM and MA. MA patients exhibit hypersensitivity to nitric oxide similar to patients with MO, but the effect of CGRP in MA patients has not been studied yet. Furthermore, we hypothesized that FHM may be seen as a part of the migraine spectrum, and we would therefore expect a similar response to known migraine triggers across subtypes.

Despite these limitations, our study was conducted in genetically well defined patients, which strengthens the outcome and suggests that the FHM genotype does not confer hypersensitivity to known migraine triggers.

AUTHOR CONTRIBUTIONS
Biostatistical analysis was conducted by J.M.H. and M.A.

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REFERENCES
27. Salmon AM, Damaj MI, Marubio LM, et al. Altered neu- 
roadaptation in opiate dependence and neurogenic inflam- 
matory nociception in alpha CGRP-deficient mice. Nat 
28. Dalkara T, Zervas NT, Moskowitz MA. From spreading de- 
pression to the trigeminovascular system. Neurol Sci 2006;27 
supp 2:S86–S90.
29. Mogil JS, Miermeister F, Seifert F, et al. Variable sensitiv- 
ity to noxious heat is mediated by differential expression of 
the CGRP gene. Proc Natl Acad Sci USA 2005;102: 
12938–12943.
30. Levy D, Barstein R, Strassman AM. Calcitonin gene-related 
peptide does not excite or sensitize meningeal nociceptors: 
implications for the pathophysiology of migraine. Ann Neu- 
31. Thomsen LL, Iversen HK, Brinck TA, Olesen J. Arterial su-
persensitivity to nitric oxide (nitroglycerin) in migraine suffer-
32. Petersen KA, Birk S, Lassen LH, et al. The CGRP-
antagonist, BIBN4096BS does not affect cerebral or sys-
temic haemodynamics in healthy volunteers. Cephalalgia 
33. Lykke Thomsen L, Kirchmann Eiziken M, Faerch Romer 
34. Arulmani U, Gupta S, Maassenvandenbrink A, et al. Exper-
imental migraine models and their relevance in migraine ther-
trinitrate induces attacks of migraine without aura in sufferers 
36. Afridi SK, Kaube H, Goadsby PJ. Glyceryl trinitrate triggers pre-
37. Thomsen LL, Krause C, Iversen HK, Olesen J. A nitric oxide 
donor (nitroglycerin) triggers genuine migraine attacks. Eur 
analysis of three FHM genes in 39 sporadic patients 
with hemiplegic migraine. Neurology 2007;69:2170– 
2176.

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Neurology 71 September 9, 2008
Habituation of evoked responses is greater in FHM1 and FHM2 patients than in normal controls: a contrast with the common forms of migraine.

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Abstract

Familial hemiplegic migraine is a migraine subtype with mutations in a single gene causing a dysfunction in ion channels (FHM1,3) or pumps (FHM2) which in transgenic animal models may lead to neuronal hyperexcitability and decreased threshold for cortical spreading depression. The common forms of migraine with or without aura are characterised interictally by a habituation deficit of cortical and subcortical evoked responses which has been attributed to neuronal dysexcitability. FHM and the common forms of migraine are thought to belong to a spectrum of migraine phenotypes with similar pathophysiology. As part of the EUROHEAD project, we searched therefore if an abnormal habituation pattern would also be found in FHM patients.

In 9 patients from the Danish FHM cohort (5 FHM-1, 4 FHM-2, mutations R583Q, C1369Y and R763C, R202Q) and 7 Danish healthy volunteers (HV) we measured habituation of visual evoked potentials (VEP), auditory evoked potentials including intensity dependence (IDAP) and the nociception-specific blink reflex (nsBR).

FHM patients had a more pronounced habituation during VEP (p=0.025) and nsBR recordings (p=0.023) than HV. There was no significant difference for IDAP, but the slope tended to be steeper in FHM, despite quasi normal habituation at 90 dB. Pain thresholds (nsBR) were significantly higher in FHM patients (p=0.039).

Contrary to the common forms of migraine, FHM patients are not characterized by a deficient, but rather by an increased habituation in cortical/brain stem evoked activities. These results suggest that there may be some striking pathophysiological differences between FHM and the common forms of migraine, as far as central neuronal processing is concerned.
Introduction

Familial hemiplegic migraine (FHM) is a rare autosomal dominant migraine phenotype, characterised by motor weakness as an obligatory part of the aura. It is classified in the International Classification of Headache Disorders-2nd edition as a subtype of migraine with aura (MA) (1). Up to now, mutations causing FHM have been identified in 3 genes located on chromosomes 19p13 for FHM-1 (~50% of families) (2), 1q23 for FHM-2 (20%-30%) (3) and 2q24 for FHM-3 (4). The genes code for subunits of an ion channel (FHM-1: Ca_v2.1; FHM-3: Na_v1.1) or pump (FHM-2: Na^+/K^+-ATPase α_2 subunit) and the functional consequences of their mutations are thought to be neuronal hyperexcitability and increased susceptibility to cortical spreading depression (CSD) because of either excessive synaptic glutamate release (FHM-1) or decreased removal of K^+ and glutamate from the synaptic cleft (FHM-2) or excessive extracellular K^+ (FHM-3) (see review in 5). The transgenic knock-in mice for two of the FHM-1 mutations are characterised by a decrease in CSD threshold and an increase in glutamate release (6).

There are many phenotypic similarities between FHM and the more common, probably polygenic, forms of migraine with aura (MA). Nearly always, at least three or four aura symptoms occur in FHM, typically in the temporal order: visual, sensory, motor, aphasic. Although the aura in MA is frequently only visual, it may progress to include hemiparesthesias or dysphasia. Besides a somewhat longer duration in FHM, the characteristics of the headache are similar between the two migraine forms (7). FHM and MA attacks may alternate in patients and co-exist within FHM families. Compared with the general population, FHM probands have an eight times increased risk for MA (7). Taken together, these features have led to the suggestion that FHM and MA are part of the same phenotypic spectrum and may share common pathophysiological mechanisms.

In the common forms of migraine, both in migraine with and without aura (MO), the brain and brain stem are characterised between attacks by a lack of habituation, i.e. amplitude decrease, of evoked responses or reflexes during repeated stimulation. This functional abnormality which
fluctuates in temporal relation with the migraine cycle, has been described for visual, auditory, somatosensory and nociceptive evoked cortical potentials (see review in 8), but also for the nociception-specific blink reflex (9, 10). The habituation deficit of the latter and of visual evoked potentials are correlated in the same patients which suggests a common underlying mechanism (11). The increased intensity dependence of auditory evoked cortical responses (12) is also due to a lack of habituation chiefly at the high intensity stimulations (13). Based on studies of transcranial magnetic stimulation of the visual cortex, it was suggested that the cerebral cortex is hyperexcitable in migraine (14, 15). Although this hypothesis is not supported by other electrophysiological studies (16, 17, 18, 19), neuronal hyperexcitability could in theory be responsible for the habituation deficit.

As neuronal hyperexcitability appears to be the hallmark of FHM, it seemed of particular interest to study habituation of cortical and subcortical evoked responses in FHM patients. Thanks to the EUROHEAD project, we were able to perform such a study on a cohort of 9 FHM-1 or FHM-2 patients from the Danish FHM population-based sample of which the genetic details have been reported elsewhere (20).

**Material and Methods**

**Subjects**

We studied 9 FHM patients, 5 FHM-1 bearing the R583Q or C1369Y mutations, and 4 FHM-2 with the novel R202Q or R763C mutations (4 M/ 5 F, mean age: 38; range: 20–63 years) (Tables 1 and 2) and 7 healthy Danish volunteers (3 M/ 4 F, mean age: 29; range: 28–31 years). Inclusion criteria for the patients were a diagnosis of FHM according to the criteria of the International headache Society (1) and a known mutation in the CACNA1A and ATP1A2 genes. The study was part of the EUROHEAD project, (www.eurohead.org).

The patients were recruited from a Danish population based sample of FHM patients of which the clinical (7) and genetic hallmarks have been published (20). The cohort consists of 33 FHM patients
with known mutations. Fifteen patients who had participated in other EUROHEAD studies, were contacted and asked to participate in the study. Six patients declined participation for unspecified reasons and we were thus able to record 9 out of the 33 patients (27%) of the Danish population based FHM cohort.

The T666M mutation in the CACNA1A gene, thought to be the most prevalent (21, 22, 23), was not present in any of the participating patients, but R583Q, the second most frequent mutation (24, 25) and the most prevalent Danish FHM-1 mutation (20), was represented in 4 patients (table 1). Mutations R202Q (3 subjects) and R763C (1 subject) are novel mutations of the ATP1A2 gene found in the Danish families (table 2).

Exclusion criteria for the patients were any daily medication intake except for oral contraceptives and serious somatic or psychiatric diseases. Any drugs were discontinued at least 1 week before the experiments, and all participants were thus medicine- and headache free at the time of the recordings.

The healthy volunteers had neither a personal nor a family history of migraine or of any other frequent or chronic headache disorder.

The study was approved by the Ethics Committees of the County of Copenhagen (KA 04088) and was undertaken in accordance with the Helsinki Declaration of 1964, as revised in Edinburgh in 2000. All subjects gave informed consent to participate in the study.
Data acquisition and analysis

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, 7 at the clinical neurophysiology laboratory of Glostrup Hospital. The investigators who performed the electrophysiological recordings (MB, VdP) and analysed them (MB) 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 (nsBR) in a semi-randomized order starting with either VEP or AEP. The portable equipment consisted of a folding armchair and two 80 cm high racks in which the recording/pre-amplifier devices and the monitor/visual stimulator were positioned. For the recording we used a CED (Cambridge Electronic Design®, UK) 1902 pre-amplifier and a CED micro1401 MKII interface system, for data acquisition and analysis the CED Signal 3.08 software.

For VEP, subjects were seated 1 m in front of the monitor with their left eye patched. Stimuli were presented as a checkerboard pattern of white and black squares, subtending 1 degree and 8 min of arc, at a reversal frequency of 3.1 Hz. The active electrode (sterilized needle electrode) was inserted into the scalp midline at Oz (2.5 cm above the inion), the reference electrode at Fz. A ground electrode was fixed on the forearm. Six hundred stimuli were delivered continuously and the responses averaged in 6 blocks of 100 sweeps. The N1 peak was defined as the most negative point between 60 and 90 ms after the stimulus, P1 as the most positive point following N1 between 80 and 120 ms and N2 as the most negative point following P1 between 90 and 200 ms. We focused our analysis on the N1-P1 component, as in previous migraine studies, the most reproducible abnormalities of habituation were found for this component (8). Habituation was thus calculated as the percentage change of N1-P1 amplitude between the 1st and 6th block of averaging.

The AEP was elicited by 1000 Hz tones (duration 4 ms) delivered binaurally by earphones at a frequency of 0.474 Hz at four different intensities above sensation level (50, 60, 70 and 80 dB) in a pseudo-randomized order. The active needle electrode was positioned at Cz linked earlobes as
For each intensity 120 responses were recorded. A 1st off-line averaging was performed on the total number of artefact-free recordings (‘global averaging’). In a second off-line step, the recordings were partitioned in 4 sequential blocks of 30 trials, among which at least 25 artefact-free trials were averaged (‘block averages’). We measured the N1-P2 amplitude for each stimulus intensity, N1 being defined as the most negative peak between 60 and 150 ms after the stimulus artefact, P2 the most positive peak after N1 between 120 and 200 ms. The intensity dependence of auditory evoked cortical potential (IDAP) was expressed as the slope of the amplitude/stimulus intensity function in μV/10 dB for global and block averages. Habituation was calculated as the percentage N1-P2 amplitude change between 1\textsuperscript{st} and 4\textsuperscript{th} blocks for each stimulation intensity (13, 26).

The nsBR was obtained by stimulating A\textdelta fibres with a custom-built planar concentric electrode placed on the forehead close to the supra-orbital foramen (27) delivering monopolar square pulses of 0.5 ms at a pseudo-randomized stimulus interval of 15-17 sec (DS7A stimulator, Digitimer\textsuperscript{\textregistered}). Recording surface electrodes were placed over the orbicularis oculi muscle infra-orbitally (active) and latero-orbitally (reference), with a ground electrode on the nose. For the subjects’ comfort and to avoid a too lengthy procedure, we only recorded the blink reflex on the right side. Perception and pain thresholds were determined with an ascending and a descending sequence of 0.2 mA intensity steps. The stimulus intensity for the nsBR recordings was set at 1.5 times the individual pain threshold. We recorded 5 blocks of 6 rectified EMG responses with an inter-block interval of 2 min for a total recording duration of 16 minutes. Five responses were averaged for analysis of the R2 blink reflex, as the 1\textsuperscript{st} response of each block was excluded to avoid contamination by the R3 component (27). For each of the averaged 5 blocks we determined the mean area under the curve (AUC) of the R2 component in the time window from 27 to 87 ms, as well as mean onset latencies. Habituation of the R2 blink reflex was defined as the percentage change of the R2 AUC between the 1\textsuperscript{st} and the 5\textsuperscript{th} block of recordings.
Statistical analyses

Quantitative variables were expressed as means ± SD. Because of the small sample size, all variables described in the results section were analyzed with non-parametric tests for independent samples: Mann-Whitney’s U test for between group comparisons and Wilcoxon’s test for within group comparisons. Median and Z values are given in the text. Results were considered significant at \( p \leq 0.05 \) and to show a statistical trend at \( 0.10 \geq p > 0.05 \). Correlations between the neurophysiological data and migraine intensity, duration or frequency were analyzed with Spearman’s rank correlation test. Statistical analyses were carried out using version 7.1 of the STATISTICA program for Windows (StatSoft, Inc°, Tulsa, USA).

Results

Visual Evoked Potentials (VEP)

The N1, P1 and N2 components were clearly identified in 15 out of 16 recordings fig. 1); the recording of 1 healthy subject was discarded because of its poor quality. VEP latencies did not differ significantly between sequential blocks or between groups of subjects (table 2).

First block N1-P1 amplitudes tended to be higher in FHM-2 subjects than in healthy volunteers (HV), but this difference was not significant (\( Z=-0.63; p=0.52; \) median HV= 4.92μV, median FHM-2=5.65μV) (table 2; fig. 2).

In both FHM-1 and FHM-2 subjects, N1-P1 amplitudes decreased progressively during the 3 minutes of visual stimulation (habituation slope = -0.15 and -0.28 respectively) (table 2; fig 2). In the 6th block, the amplitude decrease relative to the 1st block was \(-12.58\% \) in FHM-1 (\( p=0.04 \)) and \(-14.87\% \) in FHM-2 (\( p=0.14 \)). In HV, N1-P1 amplitude remained almost constant along the complete visual stimulation period (slope= 0.03) with a 6th block amplitude change relative to the first block of 0.96%. The difference in habituation was significant between healthy subjects and the total group of patients (\( Z = 2.23; p = 0.02; \) median HV=5.02%, median FHM= -16.61%) and
between the former and FHM-2 patients ($Z=2.13; p=0.03; \text{median } \text{FHM-2}= -18.73\%$); it tended to be significant between control subjects and FHM-1 patients ($Z=1.64; p=0.10; \text{median } \text{FHM-1}=-8.33\%$).

There was no correlation between VEP N1-P1 habituation and clinical features like attack frequency, intensity or duration.

**Auditory evoked potentials (AEP)**

In all recordings the N1 and P2 AEP components were clearly identified (fig. 3) and their latencies were not significantly different between groups.

When N1-P2 amplitudes were compared in global averages of 120 responses at each stimulation intensity, there were no significant differences between groups, even though in FHM-2 patients they tended to be lower (table 3; fig 4). The lower amplitude in FHM-2 was also found in the 4 blocks of 30 responses and the difference with HV tended to be significant in blocks 1 and 2 at the 60 dB stimulation intensity (block 1: $Z=1.89 \ p = 0.058; \text{median } \text{HV}= 11.67\mu\text{V}, \text{median } \text{FHM-2}= 6.74\mu\text{V}$). Intensity dependence slopes (IDAP) calculated on global and block averages, though slightly steeper in patients, were not significantly different between FHM and control subjects, nor within the two FHM genotypes (fig. 4).

AEP N1-P2 amplitudes decreased during stimulation at the various intensities, i.e. habituated, in HV (table 3). At the 60 dB stimulation intensity, N1-P1 amplitude increased between the 1\textsuperscript{st} and the 4\textsuperscript{th} block, i.e. potentiated, in FHM subjects, a potentiation which was more marked (27.74\%) in FHM-1 subjects. This difference tended to be significant for FHM-1 subjects (HV/FHM-1: $Z=-1.70; p=0.088$) and for the whole group of FHM subjects (HV/FHM: $Z=-1.32; p=0.10$), but not for FHM-2 (HV/FHM2: $Z=-0.38; p=0.70$).

At higher stimulation intensities, however, the potentiation in FHM patients at 60 dB was progressively replaced by a habituation which at 90 dB exceeded in FHM-2 (-14.91\%) that of HV (-1.77\%) (table 3). The difference of the amplitude change between 60 and 90dB was significant in
the total FHM group (Z = 1.95; p = 0.05; median change at 60 dB=18.30%, at 90 dB=-9.10%) and tended to be significant in the FHM-1 group (Z=1.75; p = 0.07; median change at 60 dB=18.30%, at 90 dB=-9.10%).

**Nociception-specific blink reflexes (nsBR)**

No measurable recordings of the nsBR could be obtained in 3 healthy volunteers and 1 FHM-1 subject (patient 3, R583Q mutation).

Mean perception thresholds tended to be higher in FHM subjects than in healthy volunteers (HV/FHM-1: Z=-1.70; p=0.088, median HV=0.33μV, median FHM-1=0.61μV; HV/FHM-2: Z=-1.04; p=0.29, median FHM-2=0.53μV; HV/FHM: Z=-1.69; p=0.09, median FHM=0.61μV) (table 5). Mean pain thresholds were significantly higher in the FHM group than in the control group (Z=-2.64; p = 0.039, median HV=0.90μV, median FHM=2.10μV). The mean stimulus intensity used for studying the nsBR was thus higher in the two FHM groups (table 4).

The mean area under the curve (AUC) of the 1<sup>st</sup> block of nsBR averages did not differ significantly between groups of subjects, nor did nsBR latencies (table 4).

Over the subsequent blocks of 5 responses, the decrease, i.e. habituation, of the nsBR AUC was markedly more pronounced in both FHM groups than in the control group (fig. 5 & 6). The amplitude change in the 5<sup>th</sup> block relative to the 1<sup>st</sup> block was on average -18.0% in controls (median -12.86%), compared to -51.7% in FHM-1 (median -64.32%, Z=1.41; p = 0.15) and -54.8% in FHM-2 subjects (median=-51.17%, Z=2.31; p = 0.02) (table 4).
Discussion

Because of the small sample size and the study protocol with recordings made with a portable device at the subjects’ home, the results presented here must be taken with caution and need to be confirmed on a larger samples. They offer, nonetheless, some insight into the similarities and differences in pathophysiology between familial hemiplegic migraine and the common forms of migraine, and contribute indirectly to the understanding of the cerebral mechanisms causing some of the interictal electrophysiological abnormalities in the latter.

If, as mentioned in the introduction, neuronal hyperexcitability were the culprit for the interictal habituation deficit of evoked responses in the common forms of migraine, and if the latter and familial hemiplegic migraine (FHM) belonged to the same pathophysiological spectrum, one would expect that FHM patients might present at least some of the electrophysiological abnormalities found in migraine with/without aura (8). The results presented here provide little support for this hypothesis. We will discuss them in sequence.

The most reproducible abnormalities of visual evoked potentials in migraine without/with aura compared to healthy volunteers are lack of habituation or even potentiation during repeated stimulation and low amplitude of the averaged responses obtained after a small number of responses (1st block) (28, 29). We found on the contrary that VEP habituation was significantly superior in FHM patients than in healthy subjects and that 1st block amplitude was greater. Admittedly, habituation of the VEP was not very pronounced in the Danish controls compared to other normative subject groups. However, the amplitude decrease between 1st and 6th blocks in Danish FHM subjects (12-14%) was percentage wise within the range of that commonly found in healthy subjects (see 28, 29).

Regarding auditory evoked potentials, there were overall no major significant differences between FHM subjects and healthy volunteers. In particular, the stronger intensity dependence characteristic of common migraineurs (12, 13) was not found in FHM although the amplitude-stimulus intensity slope was slightly steeper in patients. For instance, this slope was 1.5 μV/10 dB in a clinical sample
of migraine without aura patients (13) while it was 0.06 μV/10 dB in Danish FHM subjects. Ambrosini et al. (13) have shown that in healthy controls the N1-P2 AEP component habituates during stimulus repetition at each of the four stimulation intensities which contrasts with a potentiation in migraine without aura patients. Whereas a similar result was found here in Danish healthy subjects, i.e. habituation, the pattern in Danish FHM patients is at the opposite of that described in migraine without aura, since the auditory evoked response habituates at all stimulation intensities, except at the 60 dB stimulation. At the highest intensity, the AEP amplitude decreased numerically even more between 1st and 4th blocks in FHM patients than in healthy controls.

Similarly, the pattern of habituation of the nociception-specific blink reflex in FHM subjects is at odds with that found in the common forms of migraine. In the latter, habituation of the nsBR is more than 50% smaller in migraine without aura patients and in subjects “at risk” having a 1st degree relative migraineur than in healthy controls with the stimulation protocol used here (10). Surprisingly in our study, nsBR habituation was greater in FHM subjects than in controls. Again this difference may be exaggerated by the fact that Danish healthy controls had a weak habituation compared to other normative cohorts (10), notwithstanding the fact that only subjects without a family history of migraine at any degree were carefully selected. As for the IDAP discussed above, however, the ± 50% nsBR habituation in FHM is well within the range of previous normative values. Another finding of the blink reflex study was the increased pain threshold upon the supraorbital electrical stimulation in the FHM cohort. This contrasts with decreased pain thresholds reported interictally in the common forms of migraine, either bilaterally in adults at the level of the cornea (30), in migrainous children (31) or on the side of strictly unilateral migraine (32). We have no explanation for this difference, but interestingly increased pain thresholds were also found in cluster headache patients between attacks where they were attributed to increased activation of central anti-nociception systems (33). Whether this may also be an explanation for our findings in FHM remains to be determined.
There were subtle differences in some results between FHM-1 and FHM-2 subjects. For instance, in the latter 1st block VEP N1-P1 amplitude tended to be greater while AEP amplitude was smaller. The potentiation of AEP at the 60 dB stimulation was more pronounced in FHM-1 than in FHM-2. As these differences may be spurious because of the small number of subjects in each subgroup, we will not discuss them further.

As mentioned in the introduction, the functional consequences of the mutated genes CACNA1A in FHM-1 (gain of function) and ATP1A2 in FHM-2 are thought to increase neuronal excitability, glutamate release and susceptibility for cortical spreading depression thought to be neuronal hyperexcitability and increased susceptibility to cortical spreading depression (5, 6, 34). It has been suggested that interictal neuronal hyperexcitability might also be a culprit in the common forms of migraine (14, 15) and that cortical spreading depression could be in all forms of migraine the underlying pivotal mechanism which would activate the trigeminovascular system (35) and be the major target of preventive anti-migraine drugs (36).

The habituation deficit found between attacks in migraine without or with aura can in theory be due to homosynaptic facilitation and/or decreased intracortical inhibition both causing cortical hyperexcitability (see reviews in 8 and 37). However, as discussed in detail elsewhere (19) this does not fit with the low amplitude of evoked responses obtained after a small number of stimuli, i.e. 1st block amplitude, nor with the observation that transcranial magnetic stimulations which activate the underlying visual cortex normalize habituation in migraineurs (16). An alternative mechanism might explain the lack of habituation: a reduced preactivation level of sensory cortices due to a dysfunction of thalamo-cortical loops and a decrease of serotonergic control of these loops. Recent data favouring this hypothesis come from the study in migraine patients of high frequency bands in cortical evoked potentials (see review in 19) and cortical serotonin synthesis (38). The results obtained here indicate that the habituation pattern of evoked responses in FHM patients is clearly not the one found in most patients suffering from migraine without or with aura. Habituation
is normal or supra-normal in FHM compared to Danish controls. Moreover, amplitude in the 1st block of averages tends to be greater, not smaller, in FHM subjects. This indirectly suggest that hyperexcitability of cortical neurons which appears to be an indisputable consequence of the FHM-1 and FHM-2 mutations in transgenic mice (6, 34) is not per se responsible for the habituation deficit in the common forms of migraine. Neuronal hyperexcitability could, however, explain the increased amplitude of the 1st block of visual evoked potentials in FHM (chiefly FHM-2) and the potentiation of auditory evoked potentials at the lowest stimulation intensity (mainly FHM-1).

Alternatively, one cannot exclude that in FHM an increase in cortical inhibitory mechanism might compensate between attacks for the genetically-determined increased neuronal excitability, in particular in the hemisphere usually involved during attacks. This is compatible with some transcranial magnetic stimulation studies showing decreased or normal excitability of the motor cortex in FHM patients (39, 40) and would explain their normal habituation pattern. Our study is not the only one that was unable to demonstrate in FHM the same pathophysiological abnormalities as in the common forms of migraine. A recent study of FHM-2 subjects from the same Danish families as those investigated here showed that these subjects do not share hypersensitivity to nitric oxide with the common migraine patients (41).

Taken together, and as expected from the different genotypes of the common forms of migraine mainly characterised by the association of a number of susceptibility genes, our results support the concept that various pathophysiological aspects differ between FHM and migraine without/with aura, including cortical and brain stem responsiveness. As a working hypothesis to explain these differences, we propose that, contrary to FHM where an intrinsic abnormality of cortical excitability is likely to be the culprit, the common forms of migraine are characterised by a primary brain stem dysfunction of the monoaminergic innervation of thalamus and sensory cortices which leads secondarily to a thalamo-cortical dysrhythmia and cortical hyperresponsiveness with reduced habituation (19). This hypothesis can be tested by the study of evoked cortical and brain stem responses in transgenic FHM mice and by comparing cortical innervation and activation patterns.
with functional neuroimaging methods between FHM and common migraine patients. For instance, a PET study of cortical serotonin synthesis, known to be decreased interictally in migraine without aura (38), would be of major interest in FHM patients.

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References


Legends to figures

Figure 1: illustrative recordings of PR-visual evoked potentials (VEP) in a healthy subject (HV), FHM-1 and FHM-2 patient. Six sequential blocks of 100 averaged responses are shown and the N1, P1 and P2 VEP components are identified. Vertical lines indicate the amplitude of the N1-P1 component in 1st and 6th blocks. Habituation can be seen in all three subjects.

Figure 2: Histogram of the mean amplitude (μV) changes of the N1-P1 component of visual evoked potentials over 6 sequential blocks of 100 averages. HV: healthy subjects (n=6), FHM: all FHM patients (n=9); FHM-1 (n=5); FHM-2 (n=4).

Figure 3: illustrative recordings of cortical auditory evoked potentials (AEP) at the 90 dB stimulation intensity in a healthy subject (HV), FHM-1 and FHM-2 patient. Four sequential blocks of 30 averaged responses are shown and the N1 and P2 AEP components are identified. Vertical lines indicate the amplitude of the N1-P2 component in 1st and 4th blocks. Habituation can be seen in all three subjects.

Figure 4: histogram of mean AEP amplitudes at the 4 increasing intensities of stimulation in healthy subjects (HV: n=7), all FHM (n=9), FHM-1 (n=5) and FHM-2 (n=4) patients. There is only a slight difference in the amplitude-intensity function slopes (shown in μV/10 dB) between the groups.

Figure 5: : illustrative recordings of nociception-specific blink reflexes in a healthy subject (HV), FHM-1 and FHM-2 patient. Blocks 1 and 5 of 5 averaged responses are displayed. There is a clear cut habituation between the 1st and the 5th block, more so in FHM-2 and FHM-1 than in HV.

Figure 6: histogram of the mean areas under the curve (AUC: μsecxμsec) in the 5 blocks of averages in the four subject groups. Note that the habituation is more pronounced in FHM patients than in healthy subjects (HV).
AEP: 90 dB

1st block

2nd block

3rd block

4th block

HV

FHM-1

FHM-2
AEP N1P2 amplitude - µV

- FHM -1 slope: 0.06 ± 0.06
- HV slope: 0.02 ± 0.07
- FHM -2 slope: 0.06 ± 0.07
- FHM
For Peer Review

0,0
0,1
0,2
0,3
0,4
0,5
0,6
1 2 3 4 5
blocks

ns BR Area - µV x msec

- FHM1
- HV
- FHM2
- FHM

0,0
0,1
0,2
0,3
0,4
0,5
0,6
1 2 3 4 5
blocks
Table 1: Pheno- and genotype of the 9 FHM-1 patients

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<th>Subj.#/ Age / Sex</th>
<th>Mutation</th>
<th>Frequency</th>
<th>Duration</th>
<th>Character / intensity</th>
<th>Vomiting/Nausea/ Photophobia/Phonophobia</th>
<th>Migraine with aura</th>
<th>Migraine without aura</th>
<th>Ataxia</th>
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<tr>
<td>9/20/F</td>
<td>R763C</td>
<td>&lt; 1 / year</td>
<td>2-3 h</td>
<td>throb / 10</td>
<td>yes / yes / yes / yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Table 2: N1-P1 component of visual evoked potentials: peak-to-peak amplitudes (μV) in the 6 blocks of 100 averaged responses, percentage changes in amplitude between 1st and 6th block, peak latencies. (*: P ≤ 0.05; °: 0.10 ≥ P > 0.05)

<table>
<thead>
<tr>
<th>Block</th>
<th>HV (n = 6)</th>
<th>FHM-1 (n = 5)</th>
<th>FHM-2 (n = 4)</th>
<th>FHM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.90±1.44</td>
<td>4.74±0.99</td>
<td>6.39±3.46</td>
<td>5.47±2.39</td>
</tr>
<tr>
<td>2</td>
<td>5.01±1.07</td>
<td>4.77±1.08</td>
<td>6.46±3.95</td>
<td>5.52±2.68</td>
</tr>
<tr>
<td>3</td>
<td>4.61±1.25</td>
<td>4.41±1.44</td>
<td>5.85±3.31</td>
<td>5.56±2.39</td>
</tr>
<tr>
<td>4</td>
<td>5.14±1.57</td>
<td>4.49±1.00</td>
<td>5.75±3.42</td>
<td>5.05±2.31</td>
</tr>
<tr>
<td>5</td>
<td>5.19±2.00</td>
<td>4.04±1.26</td>
<td>5.06±2.77</td>
<td>4.49±1.99</td>
</tr>
<tr>
<td>6</td>
<td>4.93±1.53</td>
<td>4.10±0.74</td>
<td>5.31±2.75</td>
<td>4.64±1.88</td>
</tr>
</tbody>
</table>

Amplitude change between 1st and 6th block

<table>
<thead>
<tr>
<th>Percentage change 6th/1st</th>
<th>HV (n = 6)</th>
<th>FHM-1 (n = 5)</th>
<th>FHM-2 (n = 4)</th>
<th>FHM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.96±10.64</td>
<td>-12.58±10.74°</td>
<td>-14.87±10.83*</td>
<td>-13.69±10.16*</td>
<td></td>
</tr>
</tbody>
</table>

Slope of change

<table>
<thead>
<tr>
<th>Slope of change</th>
<th>HV (n = 6)</th>
<th>FHM-1 (n = 5)</th>
<th>FHM-2 (n = 4)</th>
<th>FHM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03±0.21</td>
<td>-0.15±0.15</td>
<td>-0.28±0.21</td>
<td>-0.21±0.18</td>
<td></td>
</tr>
</tbody>
</table>

Mean VEP latencies

<table>
<thead>
<tr>
<th>VEP</th>
<th>HV (n = 6)</th>
<th>FHM-1 (n = 5)</th>
<th>FHM-2 (n = 4)</th>
<th>FHM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>69.96±3.33</td>
<td>71.84±6.81</td>
<td>74.20±12.00</td>
<td>72.89±8.87</td>
</tr>
<tr>
<td>P1</td>
<td>97.03±5.00</td>
<td>101.14±5.00</td>
<td>98.56±7.32</td>
<td>100.00±5.87</td>
</tr>
<tr>
<td>N2</td>
<td>127.79±7.64</td>
<td>128.52±15.63</td>
<td>128.17±17.75</td>
<td>128.37±15.50</td>
</tr>
</tbody>
</table>
Table 3: N1-P2 component of auditory evoked potentials: amplitudes (μV) in global averages, sequential blocks of 30 responses and amplitude change between 1\textsuperscript{st} and 6\textsuperscript{th} block. (*: \( P \leq 0.05 \); °: \( 0.10 \geq P > 0.05 \))

<table>
<thead>
<tr>
<th>Stimulation intensity</th>
<th>AEP N1-P2 amplitudes</th>
<th>HV (n = 7)</th>
<th>FHM-1 (n = 5)</th>
<th>FHM-2 (n = 4)</th>
<th>FHM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 dB</td>
<td>Global averages (μV)</td>
<td>8.09 ± 3.45</td>
<td>8.98 ± 1.85</td>
<td>6.21 ± 3.33</td>
<td>7.75 ± 2.82</td>
</tr>
<tr>
<td></td>
<td>Block 1</td>
<td>10.50 ± 3.04</td>
<td>9.07 ± 2.18</td>
<td>7.14 ± 2.05(*)</td>
<td>8.21 ± 2.24</td>
</tr>
<tr>
<td></td>
<td>Block 2</td>
<td>11.15 ± 3.07</td>
<td>11.09 ± 2.95</td>
<td>7.32 ± 2.99(*)</td>
<td>9.41 ± 3.42</td>
</tr>
<tr>
<td></td>
<td>Block 3</td>
<td>9.84 ± 3.16</td>
<td>10.54 ± 3.01</td>
<td>5.63 ± 2.63</td>
<td>9.14 ± 3.59</td>
</tr>
<tr>
<td></td>
<td>Block 4</td>
<td>9.51 ± 3.43</td>
<td>11.00 ± 1.58</td>
<td>7.97 ± 4.74</td>
<td>9.65 ± 3.50</td>
</tr>
<tr>
<td>Amplitude change 4\textsuperscript{th}/1\textsuperscript{st} (%)</td>
<td>-7.92 ± 23.10</td>
<td>27.74 ± 36.86(*)</td>
<td>4.42 ± 36.08(*)</td>
<td>17.37 ± 36.31(*)</td>
<td></td>
</tr>
<tr>
<td>Slope of change</td>
<td>-0.18 ± 0.96</td>
<td>0.52 ± 0.82</td>
<td>0.22 ± 0.89</td>
<td>0.39 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>70 dB</td>
<td>Global averages (μV)</td>
<td>8.92 ± 3.63</td>
<td>9.92 ± 1.63</td>
<td>7.35 ± 3.18</td>
<td>8.78 ± 2.64</td>
</tr>
<tr>
<td></td>
<td>Block 1</td>
<td>11.46 ± 4.53</td>
<td>11.33 ± 1.85</td>
<td>8.87 ± 4.05</td>
<td>10.24 ± 3.22</td>
</tr>
<tr>
<td></td>
<td>Block 2</td>
<td>11.98 ± 2.00</td>
<td>12.19 ± 2.28</td>
<td>8.04 ± 3.51</td>
<td>10.35 ± 3.14</td>
</tr>
<tr>
<td></td>
<td>Block 3</td>
<td>11.71 ± 3.90</td>
<td>11.37 ± 0.99</td>
<td>9.37 ± 4.52</td>
<td>10.49 ± 3.22</td>
</tr>
<tr>
<td></td>
<td>Block 4</td>
<td>10.33 ± 4.81</td>
<td>11.18 ± 1.78</td>
<td>8.21 ± 3.34</td>
<td>9.86 ± 3.37</td>
</tr>
<tr>
<td>Amplitude change 4\textsuperscript{th}/1\textsuperscript{st} (%)</td>
<td>-9.32 ± 9.96</td>
<td>0.81 ± 27.96</td>
<td>-3.50 ± 15.82</td>
<td>-1.11 ± 22.14</td>
<td></td>
</tr>
<tr>
<td>Slope of change</td>
<td>-0.37 ± 0.49</td>
<td>-0.13 ± 1.05</td>
<td>-0.06 ± 0.36</td>
<td>-0.10 ± 0.78</td>
<td></td>
</tr>
<tr>
<td>80 dB</td>
<td>Global averages (μV)</td>
<td>8.40 ± 4.82</td>
<td>10.17 ± 3.19</td>
<td>7.28 ± 3.98</td>
<td>8.89 ± 3.65</td>
</tr>
<tr>
<td></td>
<td>Block 1</td>
<td>12.00 ± 5.09</td>
<td>11.57 ± 3.59</td>
<td>9.94 ± 4.14</td>
<td>10.40 ± 3.84</td>
</tr>
<tr>
<td></td>
<td>Block 2</td>
<td>11.59 ± 4.02</td>
<td>12.28 ± 2.84</td>
<td>8.63 ± 3.92</td>
<td>10.66 ± 3.67</td>
</tr>
<tr>
<td></td>
<td>Block 3</td>
<td>11.25 ± 3.48</td>
<td>12.65 ± 4.53</td>
<td>8.77 ± 4.28</td>
<td>10.92 ± 4.62</td>
</tr>
<tr>
<td></td>
<td>Block 4</td>
<td>9.50 ± 2.98</td>
<td>11.82 ± 3.28</td>
<td>8.69 ± 4.52</td>
<td>10.43 ± 3.98</td>
</tr>
<tr>
<td>Amplitude change 4\textsuperscript{th}/1\textsuperscript{st} (%)</td>
<td>-13.11 ± 25.47</td>
<td>2.97 ± 5.16</td>
<td>-4.22 ± 8.03</td>
<td>-0.23 ± 7.21</td>
<td></td>
</tr>
<tr>
<td>Slope of change</td>
<td>-0.78 ± 1.20</td>
<td>0.11 ± 0.29</td>
<td>-0.06 ± 0.22</td>
<td>0.04 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>90 dB</td>
<td>Global averages (μV)</td>
<td>8.86 ± 3.28</td>
<td>10.94 ± 3.21</td>
<td>8.20 ± 5.14</td>
<td>9.72 ± 4.14</td>
</tr>
<tr>
<td></td>
<td>Block 1</td>
<td>10.67 ± 4.45</td>
<td>13.27 ± 3.58</td>
<td>10.02 ± 5.21</td>
<td>11.83 ± 4.42</td>
</tr>
<tr>
<td></td>
<td>Block 2</td>
<td>11.06 ± 3.38</td>
<td>12.87 ± 3.98</td>
<td>8.67 ± 4.85</td>
<td>11.00 ± 4.66</td>
</tr>
<tr>
<td></td>
<td>Block 3</td>
<td>9.92 ± 3.91</td>
<td>13.29 ± 3.24</td>
<td>9.58 ± 7.31</td>
<td>11.64 ± 5.40</td>
</tr>
<tr>
<td></td>
<td>Block 4</td>
<td>10.51 ± 2.71</td>
<td>11.77 ± 2.94</td>
<td>8.38 ± 5.00</td>
<td>10.26 ± 4.11</td>
</tr>
<tr>
<td>Amplitude change 4\textsuperscript{th}/1\textsuperscript{st} (%)</td>
<td>-1.77 ± 19.54</td>
<td>-9.90 ± 14.96</td>
<td>-14.91 ± 19.23</td>
<td>-12.13 ± 16.05</td>
<td></td>
</tr>
<tr>
<td>Slope of change</td>
<td>-0.16 ± 1.04</td>
<td>-0.41 ± 0.74</td>
<td>-0.40 ± 1.08</td>
<td>-0.41 ± 0.84</td>
<td></td>
</tr>
</tbody>
</table>
Table 4: Mean perception and pain thresholds (μV) during recordings of the nociception-specific blink reflex (nsBR), area under the curve (AUC: μV x msec), AUC percentage changes between 1st and 5th block and mean latencies. (*: P ≤ 0.05; °: 0.10 ≥ P > 0.05)

<table>
<thead>
<tr>
<th></th>
<th>HV (n = 7)</th>
<th>FHM-1 (n = 5)</th>
<th>FHM-2 (n = 4)</th>
<th>FHM (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right perception threshold (μV)</td>
<td>0.38 ± 0.14</td>
<td>0.61 ± 0.26°</td>
<td>0.58 ± 0.31</td>
<td>0.59 ± 0.26°</td>
</tr>
<tr>
<td>Right pain threshold (μV)</td>
<td>1.07 ± 0.64</td>
<td>1.90 ± 0.90°</td>
<td>2.72 ± 1.35°</td>
<td>2.27 ± 1.13°</td>
</tr>
<tr>
<td>Mean nsBR stimulation intensity (μV)</td>
<td>1.61 ± 0.97</td>
<td>2.85 ± 1.36°</td>
<td>4.08 ± 2.03°</td>
<td>3.40 ± 1.70°</td>
</tr>
<tr>
<td>1st Block nsBR AUC</td>
<td>0.35 ± 0.13</td>
<td>0.53 ± 0.15</td>
<td>0.32 ± 0.15</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>ns BR AUC change 5th/1st block (%)</td>
<td>-18.0 ± 9.37</td>
<td>-51.7 ± 11.70°</td>
<td>-54.8 ± 16.97°*</td>
<td>-53.5 ± 22.12°*</td>
</tr>
<tr>
<td>Latencies (ms)</td>
<td>44.11 ± 3.28</td>
<td>45.81 ± 6.94</td>
<td>47.90 ± 3.53</td>
<td>44.25 ± 6.55</td>
</tr>
</tbody>
</table>