CHAPTER 8

Genetic models of migraine

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Abstract

Migraine is a common, disabling, complex brain disorder, presenting in attacks of up to three phases: a prodromal phase, the aura phase, and the headache phase. The pathogenesis of the aura and headache phases is reasonably well understood, but it is unknown how migraine attacks are triggered. Most likely, migraineurs have a genetically determined reduced trigger threshold for migraine triggers. Identification of “threshold genes” and deciphering their function will help to unravel the triggering mechanisms for migraine attacks. Familial hemiplegic migraine (FHM) is a rare monogenic subtype of migraine with aura. Three genes have been identified for FHM. Recently, knockin mice have been generated carrying human pathogenic FHM1 mutations. These show behavioral, electrophysiological and neurobiological characteristics in line with prevailing views of migraine pathophysiology. Genetic migraine models will be useful to unravel the triggering mechanisms for migraine attacks and to identify novel migraine prophylactic targets and therapies.

Introduction

Migraine is a chronic, paroxysmal, neurovascular disorder that can start at any age and affects up to 6% of males and 18% of females in the general population. Two major forms of migraine exist: migraine without aura (MO) and migraine with aura (MA). The attack may be preceded by premonitory symptoms (prodrome) in 30% of patients. An often disabling, unilateral, throbbing headache typically characterizes attacks of MO. The headache may last 4-72 hours, is aggravated by physical activity, and accompanied by autonomic symptoms like vomiting, nausea, photophobia and phonophobia. In one-third of the migraine patients the headache phase is preceded or accompanied by transient focal neurological aura symptoms. These are usually visual but may also involve sensory disturbances, speech difficulties, and motor symptoms.

Much progress has been made in elucidating the underlying mechanisms for the aura and headache phase of migraine attacks (for reviews see1,2). The migraine aura is caused by “cortical spreading depression” (CSD), a wave of intense neuronal activity that slowly progresses over the cortex and is followed by a period of neuronal inactivity. Elevated extracellular levels of K+ and glutamate are crucial to the initiation and propagation of CSD. For the headache phase, activation of the trigeminovascular system (TGVS) plays a crucial role. The TGVS consists of the meningeal and superficial cortical blood vessels that are innervated by the trigeminal nerve. The latter projects to the trigeminal nucleus caudalis (TNC) within the brainstem which in turn projects to higher order pain centers.
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(for detailed review see Ferrari & Goadsby\(^1\)) (Figure 1). There is evidence from animal experiments, but not yet in humans, suggesting that CSD might activate the TGVS potentially linking the mechanisms for aura and headache.\(^3\) Although the mechanisms for the aura and headache are relatively well understood, hardly anything is known about how migraine attacks are initiated. Such knowledge is important to design effective, well-tolerated, prophylactic treatments.

Genetic factors play an important role in migraine pathophysiology by lowering the trigger threshold for migraine attacks. Genetic research in migraine has mainly focused on the identification of genes involved in familial hemiplegic migraine (FHM), a rare monogenic subtype of MA. FHM is considered a valid model to study molecular mechanisms involved in the common forms of migraine (for review see Ferrari & Goadsby\(^1\)). Main clinical reasons include that the aura and headache symptoms are identical (apart from the hemiparesis) and that the majority of FHM patients also have attacks of common migraine. Three genes for FHM have been identified: \textit{CACNA1A} (FHM1)\(^4\), encoding the pore-forming \(\alpha_1\)-subunit of voltage-gated neuronal \(\text{Ca}_{2.1}\) (P/Q-type) calcium channels, \textit{ATP1A2} (FHM2)\(^5\), encoding the \(\alpha_2\)-subunit of glial cell Na\(^+\),K\(^+\) pumps, and \textit{SCN1A} (FHM3)\(^6\), encoding the pore-forming \(\alpha_1\)-subunit of voltage-gated neuronal \(\text{Na}_{1.1}\) sodium channels. With the identification of these genes, the concept of FHM, and likely also common types of migraine, as ionopathies, i.e. disorders of disturbed ion transport\(^1\), has gained increasing acceptance. New opportunities have become available to generate specific, genetically sensitized models to study the triggering mechanisms of migraine attacks.

**Figure 1.** Schematic representation of events in headache pathophysiology. During cortical spreading depression (CSD) – the underlying cause of the migraine aura - K\(^+\), protons, neurotransmitters and metabolites are released and can activate perivascular trigeminal nerve endings, resulting in activation of the trigeminovascular system (TGVS) and subsequently, the trigeminal nucleus caudalis (TNC). The TNC will project to higher order pain centers such as the thalamus via the modulatory periaqueductal grey (PAG). Activation of the TGVS (and possibly CSD) induces meningeal neurogenic inflammation, resulting in central sensitization.
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Methods

Migraine is caused by disturbed cellular ion transport: molecular effects of FHM mutations

FHM1 mutations affect the function of Ca\textsubscript{2.1} calcium channels. These are expressed presynaptically by neurons throughout the brain and in the peripheral nervous system at the neuromuscular junction and are directly involved in the release of neurotransmitters. Upon depolarization of the synaptic cell membrane extracellular Ca\textsuperscript{2+} enters the presynaptic terminal through the channels, neurotransmitter-containing vesicles fuse with the synaptic membrane, and neurotransmitters are released into the synaptic cleft. The functional consequences of FHM1 mutations on single channel kinetics and whole cell Ca\textsuperscript{2+} conductance have been initially studied in cellular expression systems (oocytes, mammalian cell lines, or cultured neurons) using in vitro electrophysiological (patch clamp) techniques. FHM1 mutant channels open at more negative voltages compared to normal channels and have an enhanced channel open probability.\textsuperscript{2} This gain-of-function effect results in increased Ca\textsuperscript{2+} influx, which would predict increased neurotransmission.

FHM2 mutations in the ATP1A2 gene affect Na\textsuperscript{+},K\textsuperscript{+} pumps that are primarily expressed in neurons and glial cells. These pumps transport Na\textsuperscript{+}-ions out and K\textsuperscript{+}-ions into the cell. Importantly, astrocytic Na\textsuperscript{+},K\textsuperscript{+} pumps are also essential for the clearance of neurotransmitters and K\textsuperscript{+} from the synaptic cleft. All FHM2 mutations studied result in a loss-of-function or a kinetically altered Na\textsuperscript{+},K\textsuperscript{+} pump.\textsuperscript{5,7} Such a defect may result in a reduced uptake of ions and neurotransmitters from the synaptic cleft and accordingly an increased susceptibility to CSD.

FHM3 mutations in the SCN1A gene cause a more rapid recovery from fast inactivation of neuronal Na\textsubscript{v1.1} sodium channels following depolarization.\textsuperscript{6} As these sodium channels are crucial for the generation and propagation of action potentials, the overall effects of FHM3 mutations most likely are increased frequency of neuronal firing and enhanced neuronal excitability and neurotransmitter release.

From the cellular studies, it can be hypothesized that increased susceptibility to FHM and common types of migraine may arise from a disturbed ionic balance and concomitantly increased release of the excitatory neurotransmitter glutamate.\textsuperscript{8} FHM1 and FHM3 mutations are predicted to result in enhanced release of glutamate due to increased synaptic vesicle release and neuronal firing rate, respectively. FHM2 mutations
reduce the clearance of glutamate and extracellular K⁺ from the synaptic cleft into the glia cell leading to elevated extracellular levels of glutamate and K⁺ (Figure 2). Genetically sensitized animal models can put these hypotheses to the test.

**Transgenic mouse models of migraine**

The main advantage of knockin mouse models, carrying human mutations, is that they express the mutant gene in its most natural environment, including all transcriptional and posttranslational variations. Using a gene-targeting approach, the FHM1 R192Q mutation, previously identified in patients with pure FHM (without additional symptoms)⁴, was introduced into the endogenous Cacna1a mouse gene (chapter 2).⁹ FHM1 R192Q mice do not show any overt behavioral or anatomical abnormalities. Electrophysiological measurements of cerebellar granule cells isolated from R192Q FHM1 mice show increased neuronal calcium current, also on a whole cell level. At the neuromuscular junction, which can be considered a model synapse to study effects of Caᵥ2.1 channel mutations on transmitter release, FHM1 R192Q mice show an increased evoked and spontaneous neurotransmitter release under conditions that also occur during CSD (e.g. at low extracellular Ca²⁺ and high K⁺ levels). These changes are functional (i.e.

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**Figure 2.** Schematic representation how familial hemiplegic migraine (FHM) genes can cause increased neuronal excitability and susceptibility to CSD. All three genes are implicated in ion transport and consequences of the mutations can result in increased extracellular glutamate (Glu) and K⁺ levels. FHM1 mutations cause increased calcium influx through presynaptic channels and enhanced neurotransmitter release. FHM2 mutations predict reduced removal of Glu and K⁺ from the synaptic cleft, whereas the FHM3 mutation leads to enhanced recovery from inactivation thereby facilitating a high rate of neuronal firing. (Adapted from Sanchez-del-Rio et al. *Current Opinion in Neurology* 2006;19:294-298.)
not caused by morphological abnormalities), and are gene-dosage dependent (i.e. the abnormalities in heterozygous mice are intermediate between wild-type and homozygous mice). Electrical stimulation in FHM1 R192Q mice revealed a reduced threshold and an increased propagation velocity of CSD indicating that Ca\textsubscript{2.1} channels are important in CSD. The reduced threshold likely is due to increased glutamate levels. These results indicate that the FHM1 mice are useful models to study migraine pathophysiology \textit{in vivo}. Recently, a second FHM1 knockin mouse strain was generated by us (Chapter 4), carrying the clinically more severe S218L mutation, previously identified in patients with FHM associated with ataxia and fatal cerebral edema and coma. FHM1 S218L knockin mice exhibit ataxia similar to what can be found in patients carrying this mutation. Effects on calcium influx, neurotransmitter release and CSD were similar to what was observed for the FHM1 R192Q mice, but the changes were even more prominent, in line with the severity of the phenotype in patients with this mutation.

FHM2 and FHM3 knockin mouse models are not yet available, but will be of great interest too. Knockout mice that completely lack the Na\textsuperscript{+},K\textsuperscript{+} pump have been generated, but seem of less use since homozygous animals die at birth due to respiratory problems. The observation that in these mice, whole brain GABA and glutamate levels are increased, underscores the \textit{in vivo} importance of the FHM2 gene in the regulation of neurotransmitter homeostasis.

Relevance for Neuroscience

Over the last decade, research in the field of migraine yielded a great deal of knowledge, not only on pathophysiological mechanisms of migraine and headache, but also on the fundamental physiology of the brain. Recent studies on the consequences of Ca\textsubscript{2.1} calcium channel mutations in cellular and animal models have increased our insight into the role of Ca\textsubscript{2.1} channels in CSD and nociception and how it may cause migraine. In this respect it is relevant to realize that Ca\textsubscript{2.1} channels are expressed in all structures that have been implicated in migraine pathophysiology, including cerebral cortex, trigeminal ganglia, and brain stem nuclei involved in nociception.

The fact that CSD parameters were changed in the FHM1 mouse models confirms that Ca\textsubscript{2.1} channels are important for the initiation and propagation of CSD. Effects of mutant Ca\textsubscript{2.1} channels on downstream pathways of CSD have yet to be investigated. Is the threshold for activation of the TGVS and thereby the susceptibility for headache pain different in the transgenic mice? Ca\textsubscript{2.1} channels are also involved in the modulation of pain perception by the periaqueductal grey (PAG) and the trigeminocervical complex. Electrical stimulation of the PAG can produce migraine-like headaches in non-
migraineurs. Imaging studies show activation of brainstem nuclei before the onset of the headache phase. Both findings highlight the role of the PAG and/or other brainstem regions in migraine attacks. Future studies will show whether and how PAG functioning is changed in the genetically sensitized models.

Results coming from such models will learn us more about the current hypothesis that CSD is not only a primary cause of the aura, but may also initiate headache pain. The availability of FHM2 and FHM3 knockin mouse models for Na\(^+\),K\(^+\) pump and Na\(_v\)1.1 sodium channels will undoubtedly boost research of the role of these genes in brain function too. The unique opportunities to study CSD in genetic migraine models will lead to crosstalk with the research fields of head injury and stroke. The intriguing phenomenon of mild head trauma induced fatal excessive cerebral edema in carriers of the CACNA1A S218L mutation nicely illustrates these overlapping neurological features.\(^{11}\) Similarly, the recent observation that migraine patients are at increased risk of cerebral white matter lesions and cerebellar infarcts in a attack frequency-dependent manner suggests that migraine attacks might not be harmless and may cause brain damage.\(^{12}\) CSD induced cerebral ischemia could be one of the underlying mechanisms and can be well studied in transgenic mouse models.

Relevance for Neurology

Migraine is among the most disabling diseases. Current treatment modalities are fully satisfactorily in less than half of the patients.\(^1\) Especially specific, well tolerated and effective prophylactic treatments are dearly needed. The availability of transgenic mouse models offers the opportunity of identifying novel prophylactic treatment targets and preclinical testing of novel drugs.

The identification of migraine genes has made genetic testing of patients with FHM or sporadic forms of hemiplegic migraine possible. A major challenge is to understand the genotype-phenotype correlation. In other words, how do mutations in these genes cause migraine and associated symptoms like ataxia? Detailed analyses of genetically sensitized cellular and animal models will reveal abnormal metabolic pathways causing the disease. Especially the knockin mouse models can be instrumental for specific areas of research by pinpointing crucial mechanisms and/or evaluating intervention targets. Functional data from the three known FHM genes point towards increased extracellular neurotransmitter concentration in the brain, resulting in neuronal hyperexcitability and decreased threshold for CSD. A major question is whether it is valid to extrapolate findings in FHM to common migraine. There are strong clinical arguments that this question can be answered positively. Aside from the hemiparesis, features of the aura and headache
are identical between FHM and migraine with aura. Moreover, FHM patients and their relatives are at risk for “non-hemiplegic” typical migraine with aura. This suggests that MA and FHM are indeed part of the same clinical spectrum and that FHM and common migraine, at least in part, may share common pathways.

Since all three known FHM genes are ion transporters, it is tempting to postulate that ionic disturbances are relevant in both hemiplegic and common migraine. One study revealed subclinical cerebellar abnormalities in “normal” migraine patients. Direct convincing evidence that the CACNA1A, ATP1A2 or SCNIA genes are involved in common forms of migraine is largely lacking. However, one has to take into account that most studies were underpowered to demonstrate such an involvement.

**Future directions and avenues for therapy**

The identification of the ion transporter genes in FHM has given migraine a molecular basis and increased our understanding of the pathogenesis of the disease. Identification of additional migraine genes will contribute further to the detailed dissection of the (metabolic) pathways involved. Examples of other genes possibly involved in migraine are NOTCH3, the causative gene for CADASIL and SLC1A3, encoding the EAAT1 glutamate transporter.

The generation of genetically sensitized mouse models has opened up a whole new field of migraine research. Where research has previously concentrated on elucidating the mechanisms of CSD and intracranial nociception, genetic models will facilitate research of increased sensitivity to migraine triggers and metabolic homeostasis. In addition to treatment of acute attacks, a better understanding of how a migraine attack is triggered will be instrumental to the development of specific preventative therapies.
References
