Genetic Models of Migraine

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Migraine is a common, disabling, complex brain disorder, presenting in attacks that may have up to 3 phases: a prodromal phase, the aura phase, and the headache phase. The pathogenesis of the aura and headache phases is reasonably well understood, but the mechanism by which migraine attacks are triggered is unknown. Most likely, migraineurs have a genetically determined reduced threshold for migraine triggers. Identifying “threshold genes” and deciphering their function will help to unravel thetriggering mechanisms for migraine attacks. Familial hemiplegic migraine is a rare monogenic subtype of migraine with aura. Three genes have been identified for familial hemiplegic migraine. Recently, knock-in mice carrying human pathogenic FHM1 mutations were generated, which show behavioral, electrophysiological, and neurobiological characteristics in line with prevailing views of migraine physiological processes. Genetic migraine models will be useful in unraveling the triggering mechanisms for migraine attacks and in identifying novel migraine prophylactic targets and therapies.

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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 and migraine with aura. The attack may be preceded by premonitory symptoms (prodrome) in 30% of patients. An often disabling, unilateral, throbbing headache typically characterizes attacks of migraine without aura. The headache may last 4 to 72 hours, is aggravated by physical activity, and is accompanied by autonomic symptoms like vomiting, nausea, photophobia, and phonophobia. In one third of migraineurs, the headache phase is preceded or accompanied by transient focal symptoms of neurologic aura. These are usually visual but may also involve sensory disturbances, speech difficulties, and motor symptoms.

Much progress has been made in elucidating the mechanisms underlying the aura and headache phases of migraine attacks. 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 potassium and glutamate are crucial for the initiation and propagation of CSD. During 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, which projects into the trigeminal nucleus caudalis in the brainstem, which in turn, projects into higher-order pain centers (Figure 1). Evidence from animal experiments, but not yet in humans, suggests that CSD might activate the TGVS,

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Patients with FHM also have attacks (e.g., hemiparesis) and that most patients with this condition have a rare monogenic subtype of migraine.1 Among the main clinical reasons for this validity are that FHM1, encoding the pore-forming α1-subunit of voltage-gated neuronal Ca,2.1 (P/Q-type) calcium channels, ATP1A2 (FHM2),3 encoding the α2-subunit of glial cell sodium-potassium (Na+,K+)-pumps, and SCN1A (FHM3),4 encoding the pore-forming α1-subunit of voltage-gated neuronal Na,1.1 sodium channels. With the identification of these genes, the concept that FHM and likely other common types of migraine, are ionopathies, that is, disorders of disturbed ion transport,1 has gained increasing acceptance. New opportunities have made it possible to generate specific, genetically sensitized models to study the triggering mechanisms of migraine attacks.

**MOLECULAR EFFECTS OF FHM MUTATIONS**

Mutations of FHM1 affect the function of Ca,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. On depolarization of the synaptic cell membrane, extracellular Ca2+ 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 Ca2+ conductance were initially studied in cellular expression systems (oocytes, mammalian cell lines, or cultured neurons) using in vitro electrophysiologic (patch clamp) techniques. FHM1 mutant channels open at more negative voltages than do normal channels and have an enhanced channel open probability.2 This “gain-of-function” effect results in increased Ca2+ influx, which would predict increased neurotransmission.

FHM2 mutations in the ATP1A2 gene affect the Na+,K+-pumps that are primarily expressed in neurons and glial cells. These pumps transport sodium ions out and potassium ions into the cell. More important, astrocytic Na+,K+-pumps are also essential for the clearance of neurotransmitters and potassium from the synaptic cleft. All FHM2 mutations studied result in a “loss-of-function” or a kinetically altered Na+,K+-pump.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,1.1 sodium channels after depolarization.4 Because 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.

Based on cellular studies, it can be hypothesized that increased susceptibility to FHM and common types of migraine may arise from a disturbed ionic balance and concomitant increased release of the excitatory neurotransmitter glutamate.6,8 FHM1 and FHM3 mutations are predicted to result in enhanced release of glutamate because of increased synaptic vesicle release and neuronal firing rate, respectively. FHM2 mutations reduce the clearance of glutamate and extracellular potassium from the synaptic cleft into the glia cell, leading to elevated extracellular levels of glutamate and potassium.6,8 (Figure 2). Genetically sensitized animal models can put these hypotheses to the test.

**TRANSGENIC MOUSE MODELS OF MIGRAINE**

The main advantage of knock-in 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.9 No overt behavioral or anatomic abnormalities are seen in FHM1 R192Q mice. Electrophysiological analysis of these animals shows enhanced single-channel currents in the presence of sodium-free solutions, suggesting that the mutation affects channel function specifically. These findings support the hypothesis that the R192Q mutation is a causative factor in FHM1 and provide a model system for studying the molecular basis of human migraine.

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**Figure 1.** Schematic representation of events in headache physiologic phenomena. During cortical spreading depression (CSD), the underlying cause of the migraine aura, potassium, 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 gray (PAG). Activation of the TGVS (and possibly CSD) induces meningeal neurogenic inflammation, resulting in central sensitization.

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**Figure 2.** Genetic sensitization of animal models can put these hypotheses to the test.
trophophysiological 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 Ca,2.1 channel mutations on transmitter release, FHM1 R192Q mice show an increased evoked and spontaneous neurotransmitter release in conditions that also occur during CSD (eg, at low extracellular Ca²⁺ and high potassium levels). These changes are functional (ie, not caused by morphologic abnormalities) and are gene-dosage–dependent (ie, 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,2.1 channels are important in CSD. The reduced threshold likely is because of increased glutamate levels. These results indicate that the FHM1 mice are useful in vivo models to study migraine physiological processes. Recently, we generated a second FHM1 knock-in mouse strain (A.M.J.M. van den M., oral communication, 2006) carrying the more severe clinical mutation S218L. This had been previously found in association with ataxia, fatal cerebral edema, and coma in patients with FHM. Knock-in FHM1 mice with S218L exhibit ataxia similar to that seen in human patients carrying this mutation. Effects on calcium influx, neurotransmitter release, and CSD were similar to that observed in FHM1 R192Q mice. The changes, however, were more prominent, consistent with the severity of the phenotype in patients with this mutation.

Knock-in FHM2 and FHM3 mouse models are not yet available, but will be of great interest too. Knock-out mice that completely lack the Na⁺,K⁺ pump have been generated, but appear to be less useful because homozygous animals die at birth due to respiratory problems. The observation that in these mice, whole brain γ-aminobutyric acid and glutamate levels are increased underscores the in vivo importance of the FHM2 gene in the regulation of neurotransmitter homeostasis.

**RELEVANCE FOR NEUROSCIENCE**

During 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 physiological processes of the brain. Recent studies on the consequences of Ca,2.1 calcium channel mutations in cellular and animal models have increased our insight into the role of Ca,2.1 channels in CSD and nociception and how it may cause migraine. In this respect, it is relevant to realize that Ca,2.1 channels are expressed in all stream 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? The Ca,2.1 channels are also involved in the modulation of pain perception by the periaqueductal gray and the trigeminocephal complex. Electrical stimulation of the periaqueductal gray can produce migraine-like headaches in nonmigraineurs. Imaging studies show activation of brainstem nuclei before the onset of the headache phase. Both findings highlight the role of the periaqueductal gray and/or other brainstem regions in migraine attacks. Future studies will show whether and how periaqueductal gray functioning is changed in the genetically sensitized models.

Results based on such models will teach us more about the current hypothesis that CSD not only is a primary cause of the aura but also may initiate headache pain. The availability of FHM2 and FHM3 knock-in mouse models for Na⁺,K⁺ pump and Na,1.1 sodium channels will undoubtedly boost research into the role of these genes in brain function. The unique opportunities to study CSD in genetic migraine models will lead to information exchange with the fields of head injury and stroke. The phenomenon of fatal excessive cerebral edema induced by mild head trauma in carriers of the CACNA1A S218L mutation illustrates these overlapping neurologic features. Similarly, the recent observation that migraineurs are at increased risk of developing cerebral white matter lesions and cerebellar infarcts in an attack frequency–dependent manner suggests that migraine attacks
might not be harmless and may cause brain damage. Cerebral ischemia induced by CSD could be one of the underlying mechanisms and can be studied in transgenic mouse models.

RELEVANCE
FOR NEUROLOGY

Migraine is among the most disabling diseases. Current treatment modalities are completely satisfactory in fewer than half of the patients. Specific, well-tolerated, and effective methods of prophylaxis are needed. With the use of transgenic mouse models, novel prophylactic treatment targets and preclinical testing of novel drugs can be identified.

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. The knock-in mouse models can be useful in specific areas of research by pinpointing crucial mechanisms and/or evaluating intervention targets. Functional data on the 3 known FHM genes point to increased extracellular neurotransmitter concentration in the brain, resulting in neuronal hyperexcitability and decreased threshold for CSD. A major question that arises is whether it is valid to extrapolate findings on FHM to common migraine. Strong clinical arguments point to a positive answer to this question. Aside from the hemiparesis, FHM and migraine with aura share the common features of the aura and headache. Moreover, patients with FHM and their relatives are at risk for “nonhemiplegic” typical migraine with aura. This suggests that migraine with aura and FHM are indeed part of the same clinical spectrum and that FHM and common migraine, at least in part, may share common pathways.

All 3 known FHM genes are ion transporters. Therefore, it is tempting to postulate that ionic disturbances are relevant in the hemiplegic and common migraine. One study revealed subclinical cerebellar abnormalities in patients with a “normal” migraine. Direct convincing evidence that the CACNA1A, ATP1A2, or SCN1A gene is involved in common forms of migraine is largely lacking. Most studies, however, 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 (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), and SLC1A3, encoding the EAAT1 ( excitatory amino acid transporter 1) glutamate transporter. The development of genetically sensitized mouse models has opened up a whole new field of migraine research. Whereas previous research concentrated on elucidating the mechanisms of CSD and intracranial nociception, newer genetic models will facilitate research into increased sensitivity to migraine triggers and metabolic homeostasis. In addition to treatment of acute attacks, a better understanding of the mechanism of migraine attack triggers will help in the development of specific preventive therapies.

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