Migraine pathophysiology: lessons from mouse models and human genetics

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Migraine is a common, disabling, and undertreated episodic brain disorder that is more common in women than in men. Unbiased genome-wide association studies have identified 13 migraine-associated variants pointing at genes that cluster in pathways for glutamatergic neurotransmission, synaptic function, pain sensing, metalloproteinases, and the vasculature. The individual pathogenetic contribution of each gene variant is difficult to assess because of small effect sizes and complex interactions. Six genes with large effect sizes were identified in patients with rare monogenic migraine syndromes, in which hemiplegic migraine and non-hemiplegic migraine with or without aura are part of a wider clinical spectrum. Transgenic mouse models with human monogenic-migraine-syndrome gene mutations showed migraine-like features, increased glutamatergic neurotransmission, cerebral hyperexcitability, and enhanced susceptibility to cortical spreading depression, which is the electrophysiological correlate of aura and a putative trigger for migraine. Enhanced susceptibility to cortical spreading depression increased sensitivity to focal cerebral ischaemia, and blocking of cortical spreading depression improved stroke outcome in these mice. Changes in female hormone levels in these mice helped cortical spreading depression susceptibility in much the same way that hormonal fluctuations affect migraine activity in patients. These findings confirm the multifactorial basis of migraine and might allow new prophylactic options to be developed, not only for migraine but potentially also for migraine-comorbid disorders such as epilepsy, depression, and stroke.

Introduction

Migraine is a common, multifactorial, neurovascular disorder with major individual and societal effects.1–3 Migraine affects roughly 15% of people4 and is typically characterised by disabling episodes of severe headache accompanied by nausea, vomiting, and hypersensitivity to light, sound, and smell for up to 3 days (migraine without aura). In a third of patients, attacks might be associated with transient focal neurological aura symptoms (migraine with aura); it has been suggested that migraine with and without aura are distinct disorders (panel 1). Once a migraine attack has started, the mechanisms underlying migraine aura and headache are reasonably well understood (figures 1–3). Aura is most likely caused by cortical spreading depression, and headache by activation of the trigeminovascular system and associated release of calcitonin gene-related peptide (CGRP).5 On the basis of animal experiments, cortical spreading depression has also been proposed as a possible trigger activating the trigeminovascular system, thus providing a possible pathophysiological link between aura and headache.

Attack frequency differs widely in patients, from one per year to several per week. Half the patients have attacks at least twice a month, 25% have them at least weekly, and about 3% have chronic migraine with headaches occurring at least half the time.6,7 Thus, on average, every day at least 24 million people in the European Union and North America have migraines, making the condition one of the most disabling and expensive medical complaints worldwide.8,9

For clinical, epidemiological, and genetic studies, migraineurs are defined as people who have had at least five episodes of migraine without aura or two episodes of migraine with aura ever in their life. Clinical expression is believed to be determined by genetic factors for up to 60%,28,29 and for the remaining 40% by non-genetic endogenous (eg, age, sex-related) hormonal fluctuations, and comorbid diseases (eg, head trauma, fatigue, and changes in sleeping pattern) risk-modulating factors.

Migraine, depression, and epilepsy are comorbid conditions, in that the presence of one of these diseases increases the risk of development of one or both of the others, and vice versa.30,31 The disorders also share common treatments, as specific antiepileptic drugs are also effective in migraine and depression,32 and the antidepressant drug amitriptyline is frequently used in migraine prevention.33 Taken together, these observations suggest common mechanisms, most likely shared genetic factors, for migraine, epilepsy, and depression.

Migraineurs are also at increased risk of cerebral34–36 and myocardial37 infarction, suggesting systemic involvement of the vasculature in migraine. A genome-wide association study (GWAS) in the Women’s Health Study in over 5000 women with migraine with information on cardiovascular disease suggested that genetic factors play a part in both diseases, although no specific gene variants were identified.38 Although common genetic factors for migraine and stroke have not yet been identified at the general population level, at least two genes (NOTCH3 and TREX1) are known that cause both migraine and ischaemic stroke.

Despite our knowledge of the mechanisms after initiation of the attack, there is an unmet need for treatments to prevent migraine attacks and to stop the condition from becoming chronic. The main reasons for this dearth of options are the low understanding of how migraine attacks are triggered and initiated, which raises questions such as why do migraines so often continue to
recurrent throughout life, why in a proportion of patients do attacks recur more frequently (up to several times a week), and finally why in so many patients do attacks stop recurring at older age? In this Review, we will try to answer some of these questions by reviewing the present status of the genetics and molecular neurobiology of migraine and the growing evidence that the brains of people with migraines might be hyperexcitable and more susceptible to cortical spreading depression. These findings might open up new avenues for improved migraine prophylactic treatments and might also further the understanding of the pathology of migraine-comorbid disorders such as epilepsy, depression, and stroke.

Genetic studies in patients with migraine
GWAS can identify disease-associated gene variants and thus, more importantly, markers of novel pathways. In migraine, three large GWAS8–10 and a subsequent meta-analysis11 identified 13 susceptibility gene variants (table 1). These variants point at genes that cluster into five pathways: glutamatergic neurotransmission; synapse development and plasticity; pain sensing; metalloproteinases; and vasculature and metabolism. The ascertainment of the contribution of individual variants in these pathways is difficult because the effect sizes of the gene variants are small and the interactions are complex. For example, the protein encoded by migraine-susceptibility gene LRP1 is cleaved by a metalloproteinase that is encoded by another migraine-susceptibility gene, MMP16. A promising next step should be the analysis of gene-interaction networks, but the logistics and bioinformatics of such studies are challenging.

The association with gene variants involved in glutamatergic neurotransmission and synaptic development and plasticity is well in line with accumulating evidence from studies in monogenic migraine syndromes that cerebral hyperexcitability is due to increased glutamatergic neurotransmission which is important in the initiation and recurrence of migraine attacks. These associations were noted for migraine with and without aura,9–11 supporting the concept that subclinical cortical spreading depression might also be involved in migraine without aura.

At least two migraine-associated genes are involved in the integrity of endothelial cells (PHACTR1 and the blood vessel wall (TGFB2), providing a genetic basis for the increasing epidemiological evidence that a systemic endothelialopathy is part of the migraine spectrum.35–40 TRPM8 is involved in pain, implicating pain mechanisms in migraine pathogenesis, and PRDM16 and C7orf10 are genes involved in metabolism. Although some associations were only reported for migraine with aura or migraine without aura (table 1), whether these differential findings were due to methodological issues related to GWAS or show important molecular differences between both migraine subtypes remains to be seen.

Before the GWAS era, hypothesis-driven candidate gene association studies were the method of choice and have suggested many genes for migraine.55 However, none of these genes have also been identified in unbiased GWAS,5–11 not even when the threshold for significance was lowered to very moderate p values (p<0.0001). This observation confirms the high risk of false-positive findings in small candidate gene association studies in common genetically complex disorders.55

KCNK18, which encodes for the gene product TRESK, has been reported as a gene for migraine; however, a strong debate surrounds its relevance in migraine (panel 2).

Monogenic migraine syndromes
Four rare Mendelian brain disorders have been identified in which migraine attacks are part of a wider clinical spectrum and can be considered monogenic subtypes of migraine (table 2). These subtypes may serve as genetic models to identify and unravel the pathophysiological mechanisms potentially involved in migraine.49

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterised by recurrent subcortical infarcts,
prominent white matter hyperintensities, seizures, cognitive decline, depression, and other neuropsychiatric symptoms. Migraine, particularly with aura, is a prominent feature in more than a third of patients, often preceding the other symptoms by at least a decade. CADASIL is caused by mutations in NOTCH3, which encodes the Notch3 receptor and has a key role in vascular smooth muscle cell function in the small arteries and arterioles of the brain.

Cerebral hereditary angiopathy with vascular retinopathy and internal organ dysfunction (CHARIOT; previously called retinal vasculopathy with cerebral leukodystrophy) is a progressive systemic small-vessel disease caused by mutations in TREX1. The main features of this disease include progressive blindness due to vascular retinopathy, focal and global neurological symptoms due to cerebral mass and white matter lesions, and premature death. Additional symptoms, such as migraine and Raynaud’s phenomenon are seen in more than half of patients and often precede the other symptoms by nearly a decade. In one large Dutch family, migraine and Raynaud’s phenomenon could be linked to TREX1.

Patients with familial advanced sleep-phase syndrome (FASPS) show severe disruption of the sleep-wake-cycle and other circadian rhythms. The disease is caused by missense mutations in CSNK1D, encoding casein kinase Iδ (CK1δ), which is involved in the phosphorylation of the circadian clock protein Per2. In two independent families, a pathogenic CSNK1D mutation co-segregated in nine of 11 carriers with familial advanced sleep phase syndrome and migraine with aura.

Familial and sporadic hemiplegic migraine (FHM and SHM) are characterised by migraine attacks, which are associated with transient, half-sided motor weakness but are otherwise indistinguishable from episodes of migraine with aura. The aura, headache, and associated symptoms are identical, and attacks can be provoked by similar triggering factors and treated with many of the same drugs. In two-thirds of patients with FHM, hemiplegic attacks might alternate with episodes of migraine without motor weakness. Other features of FHM that overlap with migraine are female preponderance and increased prevalence of migraine among first-degree relatives. Patients with FHM might also have additional ictal and permanent neurological features, such as ataxia, epilepsy, cognitive impairment, and loss of consciousness.

Historically, FHM has been the main focus of genetic research in migraine and the first migraine subtype for which causative genes have been identified. This focus

![Figure 1: Migraine aura is caused by cortical spreading depression](image)

Cortical spreading depression is regarded as the electrophysiological substrate of migraine aura. It is a short-lasting, intense wave of neuronal and glial depolarisation that spreads slowly over the cortex at a rate of approximately 2–4 mm/min. The depolarisation wave is accompanied by massive transmembrane ion fluxes (e.g., Ca²⁺, Na⁺, and K⁺) along their concentration gradients, followed by a long-lasting inhibition of spontaneous and evoked neuronal activity. The biphasic electrophysiological changes are associated with an apparent initial increase and longer-lasting decrease in regional cerebral blood flow. Direct evidence that cortical spreading depression underlies migraine aura stems from functional neuroimaging studies in patients displaying similar regional cerebral blood flow changes during aura as those seen in cortical spreading depression in animal experiments. These regional cerebral blood flow changes usually start in the occipital cortex and slowly spread in frontal direction. CBF=cerebral blood flow.

![Figure 2: Migraine headache is caused by activation of the trigeminovascular system](image)

The trigeminovascular system consists of nociceptive trigeminal sensory afferents surrounding cranial blood vessels. Upon activation of these perivascular trigeminal afferents, the signal travels through the trigeminal ganglion to neurons in the trigemino cervical complex, using calcitonin gene-related peptide (CGRP) as the main neurotransmitter. The signals are then relayed to the thalamus, because all nociceptive inputs are integrated through this structure, it has been named the pain matrix of the brain. Modulation of the signal occurs through extensive connections with brainstem regions such as the periaqueductal gray and the locus coeruleus. Symptoms accompanying the headache, such as allodynia, photophobia, and phonophobia, are generated by sensitisation of neurons along the pain pathway, mainly in the trigemino cervical complex and the thalamus.
has led to FHM being the most extensively studied model for migraine. Some researchers, however, have questioned whether and to what extent FHM is a valid model for migraine (panel 3).

Genes for FHM
Three genes have been identified for FHM, all encoding ion-homoeostasis-regulating proteins that control neuronal activity via modulation of the availability of glutamate at synaptic terminals (figure 4). FHM type 1 (FHM1) is caused by missense mutations in CACNA1A on chromosome 19p13.90 This gene encodes the pore-forming α1 subunit of neuronal voltage-gated CaV2.1 (P/Q-type) calcium channels that control release of neurotransmitters at peripheral and central synapses. The Ser218Leu CACNA1A mutation is responsible for perhaps the most severe form of FHM1, in which often fatal episodes of severe cerebral oedema, seizures, and coma can be triggered by minor head trauma.91 Other CACNA1A mutations, not causing FHM1, have been associated with episodic ataxia,89 spinocerebellar ataxia type 6,92 and a combination of progressive cerebellar ataxia and epilepsy.90

FHM type 2 (FHM2) is caused by missense mutations in ATP1A2 on chromosome 1q23.93 ATP1A2 encodes the α2 subunit of Na+/K+-ATPase pumps, which in early life (during embryonic development and after birth) are primarily expressed in neurons, and in adult life in glial cells, where they help the active reuptake of glutamate from the synaptic cleft.94 Additional neurological symptoms associated with mutations in ATP1A2 might include cerebellar ataxia, epilepsy, confusion, coma, prolonged hemiplegia, and mental retardation.95

FHM type 3 (FHM3) is caused by missense mutations in SCN1A on chromosome 2q24.87 This gene encodes the α1 subunit of neuronal voltage-gated NaV1.1 sodium channels, which are crucial for the generation and propagation of neuronal action potentials.87 Several hundreds of SCN1A mutations have been associated with severe epilepsy syndromes, such as severe myoclonic epilepsy of infancy and generalised epilepsy with febrile seizures.96 Mutations in FHM1 and FHM2 genes have also been reported in sporadic hemiplegic migraine,97 often associated with symptoms such as ataxia, epilepsy, mental retardation, and cerebral oedema.98 Although caused by mutations in different genes, clinically, the phenotypes of the three FHM types are nearly identical.

Other putative genes for hemiplegic migraine
Because several FHM families could not be linked to known FHM genes,99 additional genes probably exist for FHM. SLC1A3, PRRT2, and SLC4A4 have been proposed, but based on only limited evidence.99 In one patient with migraine, alternating hemiplegia, seizures, and episodic ataxia, a heterozygous missense mutation was identified in SLC1A3, which encodes the excitatory aminoacid transporter 1 (EAAT1).100 The mutation had a dominant-negative effect, leading to decreased EAAT1 expression and substantially decreased glutamate reuptake, which is predicted to result in neuronal
Hyperexcitability. Heterozygous PRRT2 mutations have been identified in a few patients with hemiplegic migraine.103 Most of these patients also had paroxysmal kinesigenic dyskinesia. Although PRRT2 might have a role in hemiplegic migraine through dysfunction of PRRT2-mediated and SNAP25-mediated neurotransmitter release, the evidence is scarce.111 Other PRRT2 mutations have previously been identified in hundreds of individuals with paroxysmal kinesigenic dyskinesia or other paroxysmal disorders, but never hemiplegic migraine.112 Possibly, PRRT2 could act as a genetic cofactor that contributes to the risk of hemiplegic migraine.113 Finally, homozygous SLC4A4 mutations, leading to a non-functional sodium bicarbonate cotransporter NBCe1, were reported in a few patients with hemiplegic migraine and proximal renal tubular...
acidity. Some of these patients also had episodic ataxia and ocular abnormalities. The investigators hypothesised that NBCe1 dysfunction and deranged synaptic pH regulation in astrocytes could lead to neuronal hyperexcitability predisposing to migraine.

**Transgenic mouse models of FHM1**

Two knock-in mouse models of FHM1 have been generated to study the functional outcomes of FHM mutations and to identify pathophysiological mechanisms potentially involved in FHM and hence possibly also in non-hemiplegic migraine. By use of a gene-targeting approach, the human pathogenic Arg192Gln or Ser218Leu missense mutation were inserted into the mouse orthologous CaCna1a by homologous recombination. Arg192Gln was chosen because it is associated with a mild pure FHM phenotype without additional clinical features, modelling migraine as closely as possible. Ser218Leu was selected because it is associated with probably the most severe FHM1 phenotype. Comparison of functional changes in both mouse models enabled a disease severity-dependent analysis and the differentiation of possible pathways for migraine and its associated features.

**Migraine-associated features in FHM1 mice**

FHM1 mice display migraine-associated features, including hemiparesis, photophobia, head pain, and enhanced response to (experimental) jet lag, which lends support to the clinical validity of these models (table 3).

Experimentally induced cortical spreading depression caused severe hemiparesis for up to 1-5 h in FHM1 mice but only mild motor weakness lasting a few minutes in wild-type mice. The motor weakness was most pronounced in Ser218Leu mice and could also occur spontaneously in these mice, probably because of innate, increased susceptibility to cortical spreading depression.

Photophobia is difficult to test in rodents because they have a natural tendency to avoid light. With an innovative modification of the elevated plus maze test in which the safe, closed arms were brightly illuminated while the exposed unsafe open arms were kept dark, FHM1 mice avoided the light-lid arms without showing differences in the standard (anxiety) version of the test.

Assessment of headache in rodents is even more challenging because of ethical and methodological issues. Multiple behavioural measures suggestive of spontaneous unilateral head pain were reported in FHM1 mice when exposed to a new situation or to restraint stress. The most important signs of head pain were head grooming, spontaneous eye blinking and sustained single-eye closures, and changes on the Mouse Grimace Scale, a novel standardised behavioural coding system to assess pain in mice on the basis of pain severity-dependent changes in facial expression. Most importantly, these pain-measures were dose-dependently normalised by systemic administration of the antimigraine agent rizatriptan. FHM1 mice might thus potentially serve as models to test putative antimigraine drugs, although further validation tests are needed.

**Panel 2: Is KCNK18 a gene for migraine?**

Lafreniere and colleagues systematically sequenced 150 brain-expressed ion channel genes in 110 migraineurs. In one large family the authors reported a mutation in KCNK18, which had a dominant-negative effect on its gene product (TRESK) and cosegregated with migraine with aura. In a follow-up study, the same researchers identified another TRESK loss-of-function mutation (Cys110Arg), not only in migraineurs, but also in controls. Evidently, a single non-functional TRESK variant is not sufficient to cause migraine. Moreover, many people can carry deleterious gene mutations without showing any disease symptoms. If KCNK18 is a migraine gene, its role is probably restricted to only a few cases because more than 500 migraine probands tested negative for KCNK18 mutations. TRESK is still an interesting target because of its expression in trigeminal neurons and its putative role in reduction of neuronal excitability under inflammatory conditions.

**Table 2: Monogenic migraine syndromes in which episodes of migraine with aura or migraine without aura are part of their clinical spectrum.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Main clinical features</th>
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<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)</td>
<td>Recurrent subcortical infarcts, prominent white matter hyperintensities, lacunar infarcts, microbleeds, cognitive decline, dementia, and other psychiatric symptoms; episodes of migraine, particularly with aura, occur in a third of patients, often preceding the other symptoms by a decade</td>
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<td>Cerebral hereditary angiopathy with vascular retinopathy and internal organ dysfunction caused by TREX1 mutations (CHARIOT)</td>
<td>Vascular retinopathy leading to blindness, focal and global neurological symptoms due to recurring cerebral mass lesions, white matter hyperintensities, cognitive decline, depression, stroke, renal and liver dysfunction, and Raynaud’s phenomenon; episodes of migraine occur in more than half of patients, often preceding the other symptoms</td>
</tr>
<tr>
<td>Familial advanced sleep phase disorder (FASPS)</td>
<td>Sleep stages advanced by 4-5 h, including body temperature and melatonin rhythms; episodes of migraine with aura occur in all patients</td>
</tr>
<tr>
<td>Familial hemiplegic migraine (FHM) and sporadic hemiplegic migraine (SHM)</td>
<td>Migraine attacks associated with transient motor weakness, often alternating with episodes of migraine not associated with motor weakness; additional features can be cerebellar ataxia, seizures, cognitive impairment, and ictal loss of consciousness</td>
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**Table 2: Monogenic migraine syndromes in which episodes of migraine with aura or migraine without aura are part of their clinical spectrum.**
Some researchers have questioned the validity of FHM as a model for migraine, mainly because the incidence of migraine-like headaches after provocation with nitroglycerin or CGRP was lower in patients with FHM than in patients with migraine who were tested in previous studies. The nitroglycerin or CGRP provocation tests were in fact positioned as diagnostic for migraine, and patients who failed these tests were not to be considered migraineurs. However, several methodological restrictions that complicate a straightforward interpretation of the results are associated with these provocation tests. At best, the results might point to quantitative differences between FHM and migraine (ie, reduced susceptibility to any external trigger including nitroglycerin or CGRP in FHM) rather than involvement of radically different disease mechanisms. Thus, although FHM and migraine are not the same disease and overextrapolation of findings from models into a clinical setting should be avoided, nitroglycerin or CGRP provocation studies cannot be used to invalidate FHM as a useful model for the identification of possible disease pathways in migraine.

Response rates to nitroglycerin and CGRP (defined as the proportion of individuals developing migraine-like headaches a few hours after administration of either compound) in patients with FHM were compared with historical responses of patients with non-hemiplegic migraine without aura who were tested in earlier studies but were not matched for baseline attack frequency. As response rates in migraineurs vary considerably over time and across study centres (from 14% to 83%) and also seem to be highly dependent on baseline attack frequency and migraine subtype, response rates in patients with FHM should have been compared with response rates obtained in migraine patients matched for attack frequency and tested within the same study.

Response rates to nitroglycerin and CGRP are generally increased in patients with migraine without aura compared with migraineurs with aura, and in migraineurs with high attack frequency compared with migraineurs with low attack frequency. Attack frequency thus seems to be a clinical marker for susceptibility to external trigger factors such as nitroglycerin and CGRP. Patients with FHM typically have a much lower attack frequency than do patients with migraine without aura, predicting lower sensitivity and thus lower response rate to nitroglycerin or CGRP provocation. To test whether different pathways are involved in FHM compared with migraine, response rates in patients with FHM should have been compared with patients with infrequent episodes of migraine.

Response to nitroglycerin or CGRP was positioned as a diagnostic test for migraine. However, sensitivity and specificity of the nitroglycerin or CGRP provocation tests are very low, invalidating its usefulness in clinical research. Depending on the study, up to 83% of patients who were formally classified as a migraineur according to the current gold standard failed the test. Moreover, administration of nitroglycerin or CGRP could provoke cluster headache in patients with a history of cluster headaches and tension-type headache in patients with a history of tension-type headaches, showing that provocation of headache with nitroglycerin or CGRP is not specific for migraine.

In patients with migraine with aura, nitroglycerin might provoke headaches, but only rarely, if ever, auras. Nitric oxide (to which nitroglycerin is converted) inhibits FHM  ion channel function and the initiation and propagation of cortical spreading depression—the electrophysiological mechanism for aura—and motor weakness in individuals with FHM. This might be an additional explanation for the lower response rates in FHM and migraine with aura.

In summary, because of methodological restrictions, the results from the nitroglycerin or CGRP provocation studies cannot be used to invalidate FHM as a useful model for identifying disease pathways for migraine and cannot be used to lend support to a view that different mechanisms involved in both types of migraine. At best the study results might suggest that patients with FHM (and probably other migraine patients with low attack frequency, like many patients with migraine with aura) have a lower susceptibility to provocation with external trigger factors such as nitroglycerin and CGRP.

absence of sleep, sleeping in, and jet lag) are frequently reported triggers for migraine attacks, possibly because migraineurs have an inadequate adaptation mechanism. After experimental 6 h advance shifts of the light-dark cycle (eastbound jet lag), Arg192Gln mice compared with wild-type controls showed a more than two-times enhanced adjustment of behavioural wheel-running activity and electroencephalographic patterns, with an abrupt shift of electrical activity of suprachiasmatic nucleus neurons in vivo. Arg192Gln mice thus do not have the physiological retardation in circadian adaptation to phase-advance shifts, most likely due to disturbed CaV2.1 channel-dependent afferent signalling from extra-suprachiasmatic nucleus areas to the suprachiasmatic nucleus. Researchers have hypothesised that acute imbalance between different brain systems might trigger migraine attacks. This concept would be in agreement with the observation that migraine is a prominent feature of familial advanced sleep phase syndrome, which is characterised by severe disruption of circadian rhythms.
Migraine-relevant mechanisms in FHM1 mice

FHM1 mice have served as valuable models (table 3) to unravel mechanisms potentially explaining how migraine attacks are triggered and initiated, why migraine is a paroxysmal disorder; why not all brain cells are similarly affected by mutations; why migraine is more common in women, and how pain mechanisms in migraine might be activated.

**Neuron-specific effects of FHM1 mutations**

Not all neurons carrying Ca\(_{\text{v}}\)2.1 channels are equally affected by FHM1 mutations. This might explain why mutations in such a widely-expressed channel cause only localised functional changes. As shown in autapases and in ex vivo brain slices, while Ca\(_{\text{v}}\)2.1 channel-mediated excitatory neurotransmission is enhanced due to increased action potential-evoked Ca\(^{2+}\) influx and probability of glutamate release, Ca\(_{\text{v}}\)2.1 channel-mediated inhibitory neurotransmission at cortical interneuronal synapses is unaltered. As a result, the net effect of FHM1 mutations is enhanced glutamatergic neuro-excitatory activity without apparent compensatory GABA-ergic interneuronal inhibition.

Downstream effects of FHM1 mutations seem to depend on the shape of presynaptic action potentials, which in turn depend on the shape and size of the presynaptic neuron. Thus, cortical pyramidal neurons are affected but the large neurons of the Calyx of Held are not. Additionally, some modulatory mechanisms of Ca\(_{\text{v}}\)2.1 calcium channels and associated synaptic neurotransmission, such as G-protein inhibition, calcium-dependent facilitation, and expression of different auxiliary Ca\(_{\text{v}}\)2.1 subunits, are altered by FHM1 mutations. Whether these mechanisms are also altered in vivo in FHM1 mice is unknown.

Similar changes might also explain why the Arg192Gln mutation has a differential effect on different subtypes of trigeminal ganglion neurons. P/Q-type Ca\(^{2+}\) current density, voltage-dependence, and kinetics were increased in capsaicin-insensitive trigeminal neurons not innervating the dura, but not in small capsaicin-sensitive neurons innervating the dura.

**Enhanced glutamatergic neurotransmission and susceptibility to cortical spreading depression**

The threshold for in vivo induction of cortical spreading depression by cortical electrical stimulation or topical application of KCl is decreased in FHM1 mice, and cortical spreading depression frequency and propagation velocity are increased. These effects are most likely due to enhanced synaptic release of glutamate as a result of the Ca\(_{\text{v}}\)2.1 channel gain-of-function mutation selectively affecting glutamatergic excitatory neurons but not GABA-ergic inhibitory interneurons.

Cortical spreading depression threshold and velocity normalised when glutamate release at pyramidal cell synapses in cortical slices was brought back to wild-type values using sub-saturating concentrations of the P/Q-type specific Ca\(^{2+}\) channel blocker ω-agatoxin IVA.

The role of Ca\(_{\text{v}}\)2.1 channels in the modulation of neurotransmitter release and the initiation and propagation of cortical spreading depression is further highlighted by findings in Tottering and Leaner strains of mutant mice. These naturally occurring Ca\(_{\text{v}}\)2.1 channel mutants carry loss-of-function mutations in the Cacna1a gene. These mutations resulted in greatly reduced neuronal P/Q-type...
Ca²⁺ currents, impaired K⁺-induced Ca²⁺-dependent neurotransmitter release, vastly increased cortical spreading depression induction threshold and, only in Leaner mice, decreased cortical spreading depression propagation velocity.

**Mutation and phenotype severity-correlated changes**

The effects on cortical spreading depression susceptibility are much greater for the Ser218Leu mutation compared with the Arg192Gln mutation, which is well in line with its much stronger gain-of-function effect on Ca,V2·1 channels and its more striking clinical phenotype in patients. Compared with Arg192Gln mice, Ser218Leu mice have greater ease of induction and propagation of cortical spreading depression, more severe and more prolonged hemiparesis and other motor deficits, and more readily propagation of cortical spreading depression into subcortical structures, even into the hippocampus and the thalamus. Moreover, while a single stimulus would induce only a single cortical spreading depression wave in wild-type mice, one stimulus would frequently cause multiple repetitive cortical spreading depression events in Ser218Leu mice because of cortico-subcortical re-entrant waves.

**Modulation by sex hormones**

Hormonal fluctuations have a large effect on migraine activity. Migraine is far more common in women than in men, in particular during the fertile period (figure 5). Migraine attacks typically begin in puberty and frequently strike perimenstrually, temporarily disappear during pregnancy and while breastfeeding, and usually worsen during menopause to completely disappear afterwards. In line with these clinical observations, in mice natural hormone concentrations strongly modulated the functional effects of FHM1 mutations. Susceptibility for KCl-induced cortical spreading depression was lower and propagation velocity and frequency were higher in female than male mice. Female hormonal fluctuations affect migraine activity by reducing the triggering threshold only in genetically predisposed individuals.

Ca,V2·1 channels modulate trigeminovascular activity and pain transmission

Animal studies have provided evidence that Ca,V2·1 channels modulate trigeminovascular activity and pain transmission within the trigeminothalamic system. Local injection of P/Q-type, N-type, and L-type calcium-channel blockers in the periaqueductal gray of rats facilitated the in vivo increase of neuronal trigeminal nucleus caudalis activity in response to dural stimulation. Stimulation of isolated trigeminal ganglia led to higher neuronal release of CGRP in Arg192Gln mice than was noted in wild-type mice. The increased CGRP concentrations mainly affected neighbouring satellite glial cells, creating a local persistent inflammatory environment and peripheral sensitisation. This effect was evidenced by upregulation of purinergic signalling via neuronal P2X₃ and satellite glial cell P2Y receptors, larger basal release of TNFa, and greater activation of macrophages. Finally, trigeminovascular responses to nociceptive electrical stimulation of the superior

<table>
<thead>
<tr>
<th>Patients with familial hemiplegic migraine</th>
<th>Arg192Gln and Ser218Leu FHM1 mouse models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient motor weakness</td>
<td>Episodes of transient motor weakness, occurring either spontaneously (Ser218Leu) or after experimental induction of cortical spreading depression (Arg192Gln and Ser218Leu)</td>
</tr>
<tr>
<td>Headache and associated features during attack</td>
<td>Photophobia and head pain, disappearing after treatment with rizatriptan</td>
</tr>
<tr>
<td>Cerebellar signs</td>
<td>Cerebellar ataxia in Ser218Leu mice, improving after activation of Ca,V2·1 dependent K⁺ channels</td>
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<tr>
<td>Cerebellar atrophy</td>
<td>Ser218Leu mice develop Purkinje cell abnormalities</td>
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<td>Seizures</td>
<td>Seizures may occur in Ser218Leu mice, either spontaneously or after experimental induction of cortical spreading depression</td>
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<tr>
<td>Severe attacks with cerebral brain oedema</td>
<td>Coma and fatal seizures might occur in Ser218Leu mice, mainly after experimental induction of cortical spreading depression, but sometimes also spontaneously</td>
</tr>
<tr>
<td>Female preponderance and influence of female hormones</td>
<td>FHM is 2–4-times more prevalent in females, and migraine activity is highly dependent on fluctuations in female hormone levels (onset around puberty, increase during fertile period, attacks occurring perimenstrually, and disappearance after menopause)</td>
</tr>
<tr>
<td>Mutation and phenotype severity-correlated changes</td>
<td>Arg192Gln mice are models of FHM with mild transient hemiparesis, head pain and photophobia without associated symptoms, while Ser218Leu mice are not only affected more severely but also display additional symptoms, such as cerebellar ataxia, seizures and fatal cerebral oedema</td>
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FHM=familial hemiplegic migraine.

Table 3: Findings in patients with (familial or sporadic) hemiplegic migraine and in transgenic FHM1 mouse models.
Review

Figure 5: 1 year prevalence of migraine in the general population
Adapted from Lipton and colleagues, Payne and colleagues, and Peterlin and colleagues. 120 1 year prevalence of migraine is defined as the proportion of subjects from the general population who have been diagnosed with migraine with or without aura according to the diagnostic criteria of the International Headache Society (ie, having had at least two episodes of migraine with aura or at least five episodes of migraine without aura) and who had at least one migraine attack in the previous year. Before 15 years of age, the prevalence of migraine is similar in boys and girls until puberty, after which a steep rise occurs in girls and a much more gradual and small increase in boys. During the fertile period, about three times more women than men suffer from active migraine.

Mechanisms for migraine comorbid diseases
Migraine with aura is associated with increased risk of ischaemic stroke, particularly in women; migraine might even be a greater risk factor for stroke than hypertension and diabetes. Studies in FHM1 mice have suggested that neuroexcitatory mechanisms implicated in migraine might also increase vulnerability to ischaemic injury. Ischaemic peri-infarct depolarisations similar to cortical spreading depression occurred earlier and more frequently in experimental stroke in FHM1 mice than in wild-type mice, exacerbating the metabolic supply–demand mismatch. Consequently, the ischaemic viability threshold for cerebral blood flow was raised, tissue and neurological outcomes worsened, and infarcts occurred more quickly, grew larger, and were associated with higher mortality in FHM1 mice than in wild-type mice. The effect of this mutation on infarct sizes was larger in female than male mutant mice (consistent with the increased risk of stroke in female migraineurs) and in Ser218Leu than Arg192Gln mice (in line with the increased risk of stroke in female migraineurs) and who had at least one migraine attack in the previous year. Before 15 years of age, the prevalence of migraine is similar in boys and girls until puberty, after which a steep rise occurs in girls and a much more gradual and small increase in boys. During the fertile period, about three times more women than men suffer from active migraine.

Sagittal sinus were lower in FHM1 than in wild-type mice, but the dependence on activation of trigeminothalamic neurons was higher.

Mouse models for other FHM genes
A knock-in mouse model for FHM2 was generated by introducing the human Trp887Arg missense mutation in the orthologous Atp1a2 mouse gene. Mutant protein concentration in the brain was strongly decreased in heterozygous mutant mice and almost undetectable in homozygous mutants (which died immediately after birth). As in FHM1 mice, in mice with heterozygous FHM2 the in vivo cortical spreading depression triggering threshold was decreased and cortical spreading depression propagation velocity was increased. These findings are in accordance with a loss-of-function effect of FHM2 ATP1A2 mutations leading to decreased reuptake of glutamate and increased susceptibility to cortical spreading depression. A knock-in mouse model for FHM3 has yet to be generated.

A final common pathway for FHM
Although all three FHM genes encode for different proteins with distinct functions and involvement in different pathways, the seemingly diverging mechanisms ultimately converge into one common pathway: increased synaptic concentration of the excitatory neurotransmitter glutamate leading to cerebral hyperexcitability and enhanced susceptibility to cortical spreading depression (figure 4). FHM1 Ca₂⁺-dependent mechanisms might also be involved in patients with migraine and FHM with epilepsy and cerebellar ataxia. As in individuals with the Ser218Leu mutation, Ser218Leu mice exhibit cerebellar ataxia and generalised tonic-clonic seizures that usually start as myoclonic jerks and frequently result in death. The ataxia most likely is caused by irregular firing of Purkinje cells, due to mutant Ca₂⁺-1 channels lowering the threshold for somatic action potentials and dendritic Ca²⁺ spikes. Activators of Ca²⁺-dependent K⁺ channels improved motor performance by normalising Purkinje cell function.

From FHM to migraine with and without aura
FHM has been used as a model to identify possible pathways for the more common forms of migraine with aura and migraine without aura. The question now is whether and to what extent mechanisms identified for FHM are also involved in non-hemiplegic migraine. Enhanced susceptibility to cortical spreading depression is a common characteristic of transgenic mouse models for monogenic migraine syndromes (table 2), and there is growing, albeit circumstantial, evidence that FHM-like mechanisms might also be involved in patients with
non-hemiplegic migraine with aura or non-hemiplegic migraine without aura.

**FHM-like mechanisms in transgenic mice for other monogenic migraine syndromes**

Substantial progress has been made in the generation of mouse models for CADASIL, FASPS, and CHARIOT. In two different transgenic CADASIL mouse models, one overexpressing the equivalent of the human NOTCH3 R90C mutation and one in which endogenous Notch3 was knocked out, susceptibility to cortical spreading depression was enhanced. Because CADASIL NOTCH3 mutations mainly affect blood vessels, these findings seem to implicate vascular mechanisms in both cortical spreading depression and migraine. A transgenic mouse model overexpressing the human pathogenic familial advanced sleep phase syndrome Thr44Ala CSNK1D (CK1δ) mutation showed features associated with migraine, including enhanced nitroglycerin-induced aldynolalia, increased neuronal activation within the trigeminal nucleus, female preponderance, increased calcium signalling in astrocyte cultures, and disruption of circadian rhythms. Most importantly, the cortical spreading depression triggering threshold was decreased. Finally, a transgenic knock-in mouse model for CHARIOT was generated in which the human mutation was introduced into the orthologous mouse gene by use of a gene-targeting approach. Both heterozygous and homozygous CHARIOT mutant mice are viable and are being characterised at the molecular and neurobiological level.

**FHM-like mechanisms in patients with migraine**

Accumulating, although circumstantial, human evidence suggests that FHM-like mechanisms, such as enhanced susceptibility to cortical spreading depression, cerebral hyperexcitability, increased glutamatergic neurotransmission, and dysfunction of mechanisms modulating ion concentrations within the brain are also involved in migraine.

Migraine has several clinical characteristics in common with established channelopathies of the brain (eg, FHM and specific epilepsies) and muscle (eg, myotonia and periodic paralysis). These problems include intermittent episodic presentation of the clinical symptoms, with similar duration and frequency of the attacks; similar trigger factors for attacks, including emotion, stress, fatigue, hormonal fluctuations, food, and weather changes; similar sex-specific hormones, related clinical expression, with attack onset mostly around puberty and gradual disappearance after age 40 years; overlapping treatment modalities; and bidirectional comorbidity of migraine with epilepsy and depression.

Clinical experience and genetic studies suggest a dynamic clinical spectrum of hemiplegic and non-hemiplegic migraine in carriers of the FHM gene mutations. Up to two-thirds of such carriers have episodes of non-hemiplegic migraine during at least some period of their life, sometimes alternating with episodes of hemiplegic migraine. Although no evidence supports the idea that FHM or other genes regulating ion-homoeostasis are directly involved in migraine, migraine GWAS have clearly implicated gene variants involved in pathways for glutamatergic neurotransmitter release and neuronal function (table 1).

In some patients and families, migraine was associated with ion-transporter genes such as the neuronal TRESK K+ channel excitability modulating gene KCNK18, the EAAT1 glutamate transporter gene SLC1A3, and the Na+-HCO3- co-transporter NBCe1B/C pH regulating gene SLC4A4 in glial cells.

Functional tests have suggested subclinically impaired cerebellar coordination in migraineurs and single-fibre studies in migraineurs, but not in patients with FHM1, who were consistent with altered release of acetylcholine at the neuromuscular junction. As P/Q-type Ca,2-1 channels are important in both mechanisms, these observations would suggest channel dysfunction in migraine.

Inhibition of cortical spreading depression might be important in prevention and potentially in treatment of migraine attacks. All migraine prophylactic agents, despite coming from numerous different pharmacological classes, share one single mechanism of action: inhibition of cortical spreading depression and glutamate-mediated pain pathways. Moreover, two clinical trials with tonabersat, an experimental drug that inhibits cortical spreading depression in animal models, have provided preliminary evidence for prophylactic efficacy in migraine, particularly migraine with aura. Transcranial magnetic stimulation during aura, possibly blocking cortical spreading depression, prevented progression from aura to headache more frequently than did sham stimulation. Several, but not all, experimental glutamate receptor antagonists showed efficacy in proof-of-concept studies, although frequently associated with adverse events.

Finally, biochemical support comes from two studies showing increased plasma and CSF concentrations in migraine, but not tension-type headache, of glutamate and the cortical spreading depression marker matrix metalloproteinase 9 (MMP-9). Although a third study failed to show increased concentrations of MMP-9.

**Conclusions and future directions**

Migraine is one of the most prevalent, disabling, undertreated, and costly medical conditions worldwide. Research efforts are focusing on disentangling the mechanisms that trigger and initiate migraine attacks as novel targets for prophylactic treatments for migraine attacks and to prevent the transition to chronic migraine.
Clinical and genetic studies have shown that migraine is a multifactorial disorder with complex interaction between multiple predisposing genetic and non-genetic factors. Differences and fluctuations in female hormone concentrations might explain why migraine is so much more prevalent in women and why migraine activity may vary so substantially throughout life.

GWAS have identified 13 gene variants in pathways involved in glutamatergic neurotransmission and synaptic function. Translation of results from GWAS to pathophysiological mechanisms is, however, one of the biggest challenges in molecular biology because the individual gene effect sizes are small and their interactions are complex. Generation and functional characterisation of induced pluripotent stem cell lines from neuronal and glial cells derived from patients with migraine might be a promising but demanding approach. Validation of GWAS findings with such an approach has been done successfully for age-related macular degeneration.

Studies in monogenic migraine syndromes have identified mutations in six genes for migraine with large effect sizes, enabling functional analyses. Transgenic mouse models carrying human pathogenic mutations in these genes showed increased glutamatergic neurotransmission and cerebral hyperexcitability leading to enhanced susceptibility to cortical spreading depression, which is the electrophysiological substrate for aura and a putative trigger for headache. In analogy to the effects of female hormones on migraine activity, changes in female hormone concentrations in transgenic mice modulated cortical spreading depression susceptibility, as a surrogate marker for migraine susceptibility. Cortical spreading depression also increased susceptibility to experimental cerebral ischaemia, and blocking cortical spreading depression improved stroke outcome.

In conclusion, cortical spreading depression might be an important triggering mechanism for migraine attacks; female hormones might affect migraine activity through modulating cortical spreading depression susceptibility via a complex molecular interaction with migraine genes; enhanced susceptibility to cortical spreading depression might explain why migraineurs are at increased risk of stroke; and blocking of cortical spreading depression might prevent migraine attacks and might improve the risk and outcome of ischaemic stroke.

The clinical challenge is to verify the relevance of enhanced glutamatergic neurotransmission and cortical spreading depression susceptibility in migraine, and to establish the safety and efficacy of blocking these mechanisms in the prevention and treatment of attacks, and to stop them from leading to chronic migraine or stroke. A potentially important step has been the finding that the drug tert-butyl dihydroquinone blocks Ca_{2+} channel activity, normalising the cellular effects of FHM1 mutations. Results from such studies might not only benefit patients with migraine but also patients with pathophysioalogically related and frequently comorbid brain disorders such as epilepsy, depression, and cerebellar ataxia.

Search strategy and selection criteria
We searched PubMed for articles published in English between Jan 1, 1944, to May 31, 2014, with the search terms “migraine”, “migraine AND model”, and “cortical spreading depression”. We also searched reference lists of identified articles for other relevant reports. The final reference list was generated according to relevance to the topics covered in the Review.

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