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 modulated 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.

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Introduction

Migraine is a common, multifactorial, neurovascular disorder with major individual and societal effects.[1–3] Migraine affects roughly 15% of people[4] 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).[5] 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).[5] 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).[19] 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.[3,27] 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.[4,28,29]

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.[5] Clinical expression is believed to be determined by genetic factors for up to 60%,[7,30] and for the remaining 40% by non-genetic endogenous (eg, age, [sex-related] hormonal fluctuations, and comorbid diseases) and exogenous (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.[31,32] The disorders also share common treatments, as specific antiepileptic drugs are also effective in migraine and depression,[33] and the antidepressant drug amitriptyline is frequently used in migraine prevention.[34] Taken together, these observations suggest common mechanisms, most likely shared genetic factors, for migraine, epilepsy, and depression.

Migraineurs are also at increased risk of cerebral[35–38] and myocardial[39] 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.[40] 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

recur 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 GWAS[8–10] and a subsequent meta-analysis[11] 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; metallo-proteinases; 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 (*TGFBR2*), providing a genetic basis for the increasing epidemiological evidence that a systemic endothelialopathy is part of the migraine spectrum.[35–37,40] *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.[54] However, none of these genes have also been identified in unbiased GWAS,[9–11] not even when the threshold for significance was lowered to very moderate p values ($p<0{\cdot}00001$). This observation confirms the high risk of false-positive findings in small candidate gene association studies in common genetically complex disorders.[54]

KCNK18, which encodes for the gene product TRESK, has been reported as a gene for migraine;[55] 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.[66]

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is characterised by recurrent subcortical infarcts,

Panel 1: Are migraine with aura and migraine without aura distinct disorders?

Migraine with aura and migraine without aura might be distinct disorders. This hypothesis arose because distinct changes in regional cerebral blood flow were noted during aura: these changes were similar to those seen in animal models of cortical spreading depression,[6] and the relative risk (RR) of migraine was different in first-degree relatives of probands with migraine with aura (RR 3·8) versus migraine without aura (1·9).[7] However, apart from these conspicuous differences, substantial evidence supports the view that migraine with aura and migraine without aura are variable clinical expressions of largely the same genetic disease.[8–13] Clinically, almost all patients with migraine with aura also have episodes of migraine without aura, either alternating or at different phases of their life.[2] Moreover, a mix of different subtypes of migraine usually occurs in families with the disorder. In many patients, the prevailing form of migraine might change over time; for example, from attacks with aura in adolescence, to attacks without aura in midlife, and isolated auras without headache at older age. This suggests that all the relevant pathophysiological pathways are present within the same patient and only need to be switched on or off, for example, by hormonal fluctuations or other external modulating factors, which change over time. This hypothesis is supported by genetic studies showing similarities between both migraine types.[8–13] Therapeutically, prophylactic and acute migraine drugs are largely equally effective in both subtypes, suggesting that both conditions share at least some mechanisms involved in the initiation and abortion of attacks. Finally, because attacks tend to strike unpredictably, investigation of spontaneous episodes of migraine without aura during the pre-ictal or early phases of the attack is logistically challenging. Exclusion of the presence of regional cerebral blood flow changes, and thus cortical spreading depression, in attacks without aura is therefore impossible. In fact, we are aware of only one such case that was associated with substantial regional cerebral blood flow changes detected on PET, which were similar to cortical spreading depression-associated regional cerebral blood flow changes.[14] Although clinically not identical, pathophysiologically, migraine with and without aura seem more alike than different. In this Review we will use the term migraine when referring to both types of migraine, and the terms migraine with aura and migraine without aura when specifically addressing differential findings in these subtypes.

prominent white matter hyperintensities, seizures, cognitive decline, depression, and other neuropsychiatric symptoms.[60] 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.[60] 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.[67]

Cerebral hereditary angiopathy with vascular retinopathy and internal organ dysfunction (CHARIOT; previously called retinal vasculopathy with cerebral leukodystrophy)[61] is a progressive systemic small-vessel disease caused by mutations in *TREX1*.[61,62] 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.[62,68] In one large Dutch family,[69] 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*),[63,64] which is involved in the phosphorylation of the circadian clock protein Per2.[42] In two independent families,[63] 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.[65] The aura, headache, and associated symptoms are identical, and attacks can be provoked by similar triggering factors and treated and prevented with many of the same drugs.[65] 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.[65]

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

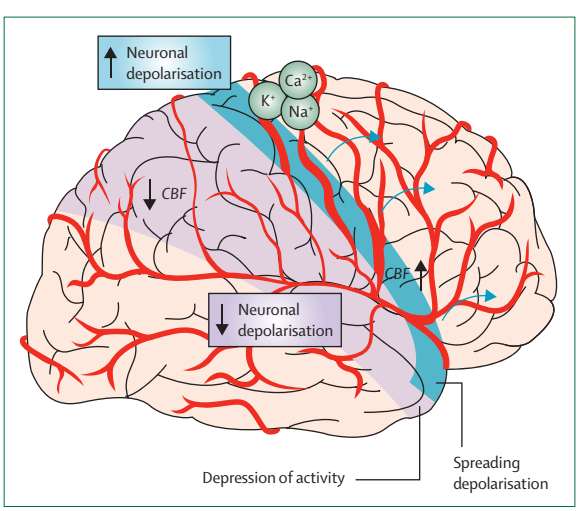

Figure 1: **Migraine aura is caused by cortical spreading depression**
Cortical spreading depression is regarded as the electrophysiological substrate of migraine aura.[15-17] In animal models, cortical spreading depression is characterised by a short-lasting, intense wave of neuronal and glial depolarisation 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 (eg, Ca^{2+}, Na^{+}, and K^{+}) along their concentration gradients, followed by a long-lasting inhibition of spontaneous and evoked neuronal activity.[15] 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.[6,18] These regional cerebral blood flow changes usually start in the occipital cortex and slowly spread in frontal direction. CBF=cerebral blood flow.

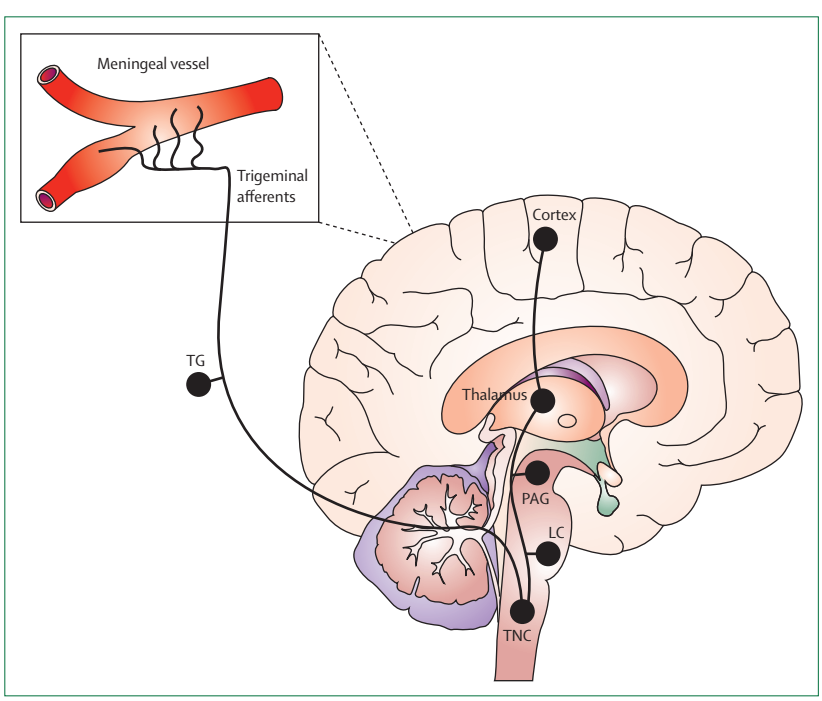

Figure 2: **Migraine headache is caused by activation of the trigeminovascular system**
The trigeminovascular system consists of nociceptive trigeminal sensory afferents surrounding cranial blood vessels. Upon activation of these perivascular trigeminal afferents, the signal travels through the trigeminal ganglion to neurons in the trigeminocervical complex, using calcitonin gene-related peptide (CGRP) as the main neurotransmitter.[19] 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.[20,21] Modulation of the signal occurs through extensive connections with brainstem regions such as the periaqueductal gray and the locus coeruleus.[20] Symptoms accompanying the headache, such as allodynia, photophobia, and phonophobia, are generated by sensitisation of neurons along the pain pathway, mainly in the trigeminocervical complex and the thalamus.[21,22] TG=trigeminal ganglion. PAG=periaqueductal gray. LC=locus coeruleus. TNC=trigeminal nucleus caudalis.

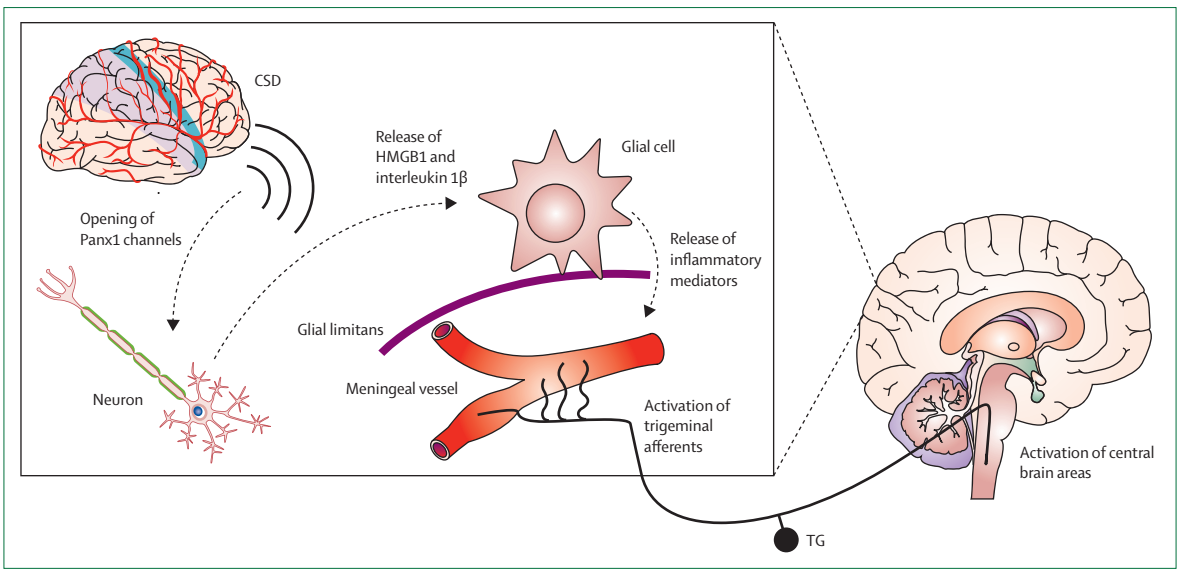

Figure 3: **Cortical spreading depression might activate the trigeminovascular system**
Experiments in rats have shown that cortical spreading depression evoked by pinprick, electrical stimulation, or KCl application might persistently activate first nociceptors that innervate the meninges and are localised within the trigeminal ganglion[23] and second (central) trigeminovascular neurons.[24,25] Delayed activation of neurons correlates with the gap between aura and headache symptoms. The activation process possibly involves opening of neuronal Panx1 channels, which in turn release HMGB1 proteins activating neighbouring astrocytes in the glial limitans surrounding meningeal blood vessels, which are innervated by the trigeminal nerve.[26] Thus, if confirmed in human beings, cortical spreading depression might not only cause the migraine aura but might also trigger the mechanisms underlying the migraine headache and associated symptoms. Adapted with permission from Karatas and colleagues.[26] CSD=cortical spreading depression. TG=trigeminal ganglion. HMGB1=high-mobility group box 1. Panx1=pannexin 1

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.[89,90] This gene encodes the pore-forming α_1 subunit of neuronal voltage-gated $Ca_v2{\cdot}1$ (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 α_2 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 α_1 subunit of neuronal voltage-gated $Na_v1{\cdot}1$ 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,[57] 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

	Chromosomal region	Gene	Description	Migraine subtype*	Study population	GWAS reference†
Glutamatergic neurotransmission						
rs1835740	8q22·1	*MTDH/AEG-1*	Astrocyte elevated gene-1 downregulates EAAT1/2, the main glutamate transporter in the brain[41]	Migraine with aura	Clinic-based	Anttila and colleagues[8]
rs11172113	12q13	*LRP1*	Lipoprotein receptor 1 interacts with glutamate (NMDA) receptors on neurons and modulates synaptic transmission[42]	Migraine without aura	Population-based or clinic-based	Chasman and colleagues,[9] Freilinger and colleagues,[10] and Antilla and colleagues[11]
rs3790455	1q22	*MEF2D*	Neuronal activity-dependent activation of the transcription factor MEF2D restricts glutamatergic excitatory synapses[43]	All migraine; migraine without aura	Population-based or clinic-based	Freilinger and colleagues[10] and Antilla and colleagues[11]
Synapse development and plasticity						
rs6478241	9q33	*ASTN2*	Member of astrotactin gene family involved in development of laminar structure of the cortex[44]	All migraine	Clinic-based	Freilinger and colleagues[10] and Antilla and colleagues[11]
rs13208321	6q16	*FHL5*	Transcription factor regulating cAMP responsive CREM/CREB proteins that influence synaptic plasticity[45]	Migraine without aura	Population-based or clinic-based	Antilla and colleagues[11]
Pain sensing						
rs10166942	2q37	*TRPM8*	Member of the TRP superfamily acting as a sensor for cold pain, expressed in sensory neurons (mostly dorsal root ganglion neurons)[46]	Migraine without aura	Population-based or clinic-based	Chasman and colleagues,[9] Freilinger and colleagues,[10] and Antilla and colleagues[11]
Metalloproteinases						
rs10504861	8q21	near *MMP16*	Metalloproteinases remodel the extracellular matrix; the protein encoded by MMP16 also cleaves lipoprotein receptor[47]	Migraine without aura	Population-based or clinic-based	Antilla and colleagues[11]
rs10915437	1p36	near *AJAP1*	AJAP1 is involved in metalloproteinase activity structuring extracellular matrix[48]	All migraine	Clinic-based	Antilla and colleagues[11]
rs12134493	1p13	near *TSPAN2*	Member of the tetraspanin family, a cell surface protein implicated in cell motility and metalloproteinase activity[49]	All migraine	Population-based or clinic-based	Antilla and colleagues[11]
rs7640543	3p24	near *TGFBR2*	Serine-threonine kinase regulating cell differentiation and extracellular matrix production[50]	All migraine	Clinic-based	Freilinger and colleagues[10] and Antilla and colleagues[11]
Vasculature and metabolism						
rs4379368	7p14	*C7orf10*	Mutations in this gene have been found in mild symptomatic forms of glutaric aciduria type III[51]	Migraine without aura	Population-based or clinic-based	Antilla and colleagues[11]
rs2651899	1p36	*PRDM16*	Transcription factor involved in brown fat development[52]	All migraine	Population-based or clinic-based	Chasman and colleagues[9] and Antilla and colleagues[11]
rs9349379	6p24	*PHACTR1*	Phosphatase and actin regulator 1 promotes synapse morphology and is implicated in endothelial cell lining[53]	Migraine without aura	Population-based or clinic-based	Freilinger and colleagues[10] and Antilla and colleagues[11]

For each pathway, the SNPs with the lowest p value mentioned in the initial GWAS are listed. *When applicable, associations specific for migraine with aura or migraine without aura are mentioned; associations to both types of migraine or which could not be linked to a specific subtype are referred to as all migraine. SNP=single nucleotide polymorphism. GWAS=genome-wide association studies. EAAT1/2=excitatory aminoacid transporters 1/2. CREM/CREB=cAMP response element/binding protein. TRP=transient receptor potential.

Table 1: **Susceptibility genes for migraine with or without aura identified in genome-wide association studies**

hyperexcitability. Heterozygous *PRRT2* mutations have been identified in a few patients with hemiplegic migraine.[101] 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.[102] Other *PRRT2* mutations have previously been identified in hundreds of individuals with paroxysmal kinesigenic dyskinesia or other paroxysmal disorders, but never hemiplegic migraine.[103] Possibly, *PRRT2* could act as a genetic cofactor that contributes to the risk of hemiplegic migraine.[102] 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

acidosis.[104] 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.[82,83] 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.

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

Lafreniere and colleagues[56] 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.[55] In a follow-up study, the same researchers identified another TRESK loss-of-function mutation (Cys110Arg), not only in migraineurs, but also in controls.[57] 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.[58] 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.[55] 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.[59]

	Genes	Main clinical features
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)[60]	*NOTCH3*	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
Cerebral hereditary angiopathy with vascular retinopathy and internal organ dysfunction caused by *TREX1* mutations (CHARIOT)[61,62]	*TREX1*	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
Familial advanced sleep phase syndrome (FASPS)[63,64]	*CSNK1D*	Sleep stages advanced by 4–5 h, including body temperature and melatonin rhythms; episodes of migraine with aura occur in all patients
Familial hemiplegic migraine (FHM) and sporadic hemiplegic migraine (SHM)[65]	FHM1: *CACNA1A*; FHM2: *ATP1A2*; FHM3: *SCN1A*	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

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

Migraine-associated features in FHM1 mice

FHM1 mice display migraine-associated features, including hemiparesis,[105,106] photophobia,[107] head pain,[107,108] and enhanced response to (experimental) jet lag,[109] 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.[105,106] 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.[83,105,106]

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.[107]

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.[107,108] 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.[108] Most importantly, these pain-measures were dose-dependently normalised by systemic administration of the antimigraine agent rizatriptan.[107,108] FHM1 mice might thus potentially serve as models to test putative antimigraine drugs, although further validation tests are needed.

Mammalian circadian rhythms are driven by the circadian pacemaker of the suprachiasmatic nucleus and are synchronised to the external 24-h light-dark cycle. After rapid advance time zone transitions (eastbound jet lag), overt circadian rhythms normally need up to 6 days to fully recover. This delayed adaptation might be a consequence of the fact that the human brain had not been exposed to jetlag until the first half of the 20th century. Perhaps this delay is part of a naturally occurring defence mechanism to protect the brain against acute imbalance in different brain systems. Acute changes in sleep patterns (such as

Panel 3: Methodological limitations associated with the nitroglycerin and CGRP provocation studies in FHM

Some researchers have questioned the validity of FHM as a model for migraine,[70] mainly because the incidence of migraine-like headaches after provocation with nitroglycerin or CGRP was lower in patients with FHM[70] than in patients with migraine who were tested in previous studies.[71] 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[44,72] were compared with historical responses of patients with non-hemiplegic migraine without aura who were tested in earlier studies [73,74] 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%)[70,75] 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 patients with migraine with aura, and in migraineurs with high attack frequency compared with migraineurs with low attack frequency.[70,72,75] 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,[74] 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.[70] 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[76] failed the test.[70,72,75] Moreover, administration of nitroglycerin or CGRP could provoke cluster headache in patients with a history of cluster headaches[77] and tension-type headache in patients with a history of tension-type headaches,[77,78] showing that provocation of headache with nitroglycerin or CGRP is not specific for migraine.

The nitroglycerin or CGRP response rates in patients with FHM were increased in individuals who also had episodes of migraine,[44] confirming the concept of a clinical spectrum of migraine subtypes (without aura, with aura, and with hemiplegic aura), which might alternate within the same patient depending on a variable interaction of genetic and non-genetic factors.

In patients with migraine with aura, nitroglycerin might provoke headaches, but only rarely, if ever, auras.[74,75] Nitric oxide (to which nitroglycerin is converted) inhibits FHM1 ion channel function[79] 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.[70,72,75]

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 volved 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,[114,115] 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.[109] Arg192Gln mice thus do not have the physiological retardation in circadian adaptation to phase-advance shifts, most likely due to disturbed $Ca_v2{\cdot}1$ 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.[63,64]

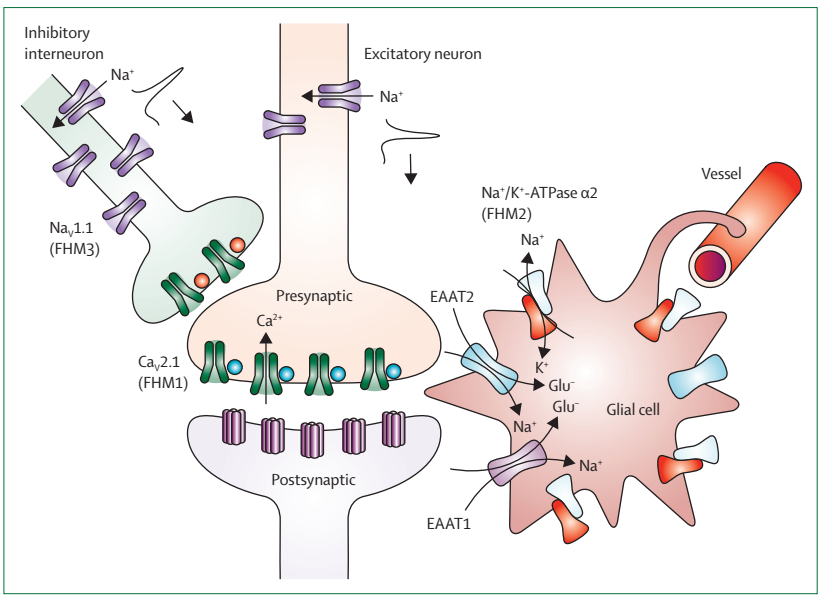

Figure 4: **A common pathway for the effects of mutations in familial hemiplegic migraine genes**
The functional effects of FHM1, FHM2, and FHM3 gene mutations all predict increased levels of glutamate in the synaptic cleft. FHM1 gain-of-function mutations in the *CACNA1A* gene encoding presynaptic $Ca_V2{\cdot}1$ calcium channels led to increased calcium influx and increased release of glutamate in the synaptic cleft by action potentials,[80,81] which was confirmed in vivo in transgenic mice.[82–84] Under normal circumstances, glutamate is taken up from the synaptic cleft into the surrounding glial cells by a glial cell Na^+/K^+ ATPase pump mechanism. FHM2 loss-of-function mutations in the *ATP1A2* gene encoding an Na^+/K^+-ATPase in the glial cell lead to reduced reuptake of glutamate leaving increased concentrations of glutamate in the synaptic cleft.[85,86] Increased activity of excitatory glutamatergic neurons is normally compensated by increased activity of inhibitory γ-aminobutyric acid (GABA)-ergic interneurons. FHM3 loss-of-function mutations in the *SCN1A* gene encoding $Na_V1.1$ sodium channels on inhibitory interneurons are predicted to lead to reduced inhibitory activity resulting in uncompensated increased excitatory neuronal activity and hence increased release of glutamate.[87,88] FHM=familial hemiplegic migraine. EAAT1=excitatory amino acid transporter 1. EAAT2=excitatory amino acid transporter 2.

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;[82,83,84,105] why migraine is a paroxysmal disorder; why not all brain cells are similarly affected by mutations;[116–118] why migraine is more common in women;[105,112] and how pain mechanisms in migraine might be activated.[119–121]

FHM1 mutation-specific changes of $Ca_V2{\cdot}1$ channels

Investigation of $Ca_V2{\cdot}1$ channel properties in cerebellar, brainstem, and cortical neurons of FHM1 mice revealed a gain-of-function of mutant $Ca_V2{\cdot}1$ channels because of increased probability of being open and shifted activation to lower voltages[82–84,113,117] and increased basal $[Ca^{2+}]_i$ concentrations.[122] The increase in P/Q-type Ca^{2+} current was much greater in Ser218Leu compared with Arg192Gln mice and in homozygous compared with heterozygous mice.[82,83] These mutation-dependent and allele dosage-dependent differences are consistent with results from analyses of recombinant human $Ca_V2{\cdot}1$ channels[80,81] and are in line with the differences in severity of the clinical symptoms between Ser218Leu and Arg192Gln mutation carriers in humans and mice, and between mice carrying one or two copies of the mutation.

Neuron-specific effects of FHM1 mutations

Not all neurons carrying $Ca_V2{\cdot}1$ 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 autapses and in ex vivo brain slices, while $Ca_V2{\cdot}1$ channel-mediated excitatory neurotransmission is enhanced due to increased action potential-evoked Ca^{2+} influx and probability of glutamate release, $Ca_V2{\cdot}1$ channel-mediated inhibitory neurotransmission at cortical interneuronal synapses is unaltered.[84] 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.[117] Additionally, some modulatory mechanisms of $Ca_V2{\cdot}1$ calcium channels and associated synaptic neurotransmission, such as G-protein inhibition, calcium-dependent facilitation, and expression of different auxiliary $Ca_V2{\cdot}1$ β-subunits, are altered by FHM1 mutations.[116,123–126] 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.[118]

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.[82,83,105] These effects are most likely due to enhanced synaptic release of glutamate as a result of the $Ca_V2{\cdot}1$ channel gain-of-function mutation selectively affecting glutamatergic excitatory neurons but not GABA-ergic inhibitory interneurons.[84] 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.[84]

The role of $Ca_V2{\cdot}1$ 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 $Ca_V2{\cdot}1$ channel mutants carry loss-of-function mutations in the *Cacna1a* gene.[127] These mutations resulted in greatly reduced neuronal P/Q-type

Ca^{2+} currents,[128] impaired K^+-induced Ca^{2+} dependent neurotransmitter release,[110] vastly increased cortical spreading depression induction threshold[110] and, only in Leaner mice, decreased cortical spreading depression propagation velocity.[110]

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,[83,105] more severe and more prolonged hemiparesis and other motor deficits,[105] and more readily propagation of cortical spreading depression into subcortical structures, even into the hippocampus and the thalamus.[106] 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[83] because of cortico-subcortical re-entrant waves.[106]

Modulation by sex hormones

Hormonal fluctuations have a large effect on migraine activity.[129] Migraine is far more common in women than in men, in particular during the fertile period (figure 5).[4,132,133] 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 differences and experimental manipulation of sex hormone concentrations strongly modulated the functional effects of FHM1 mutations. Susceptibility for KCl-induced cortical spreading depression was lower and velocity and frequency were higher in female than male mice with FHM; ovariectomy revoked and orchiectomy enhanced these sex-related differences.[105,112] Female gonadal hormones thus reinforced the enhancing effects of FHM1 mutations on cortical spreading depression susceptibility, possibly by increasing cortical hyperexcitability, whereas male hormones had the opposite effect. Importantly, these hormonal effects were only observed in mice carrying an FHM1 mutation and not in wild-type mice, supporting the multifactorial hypothesis that female hormonal fluctuations affect migraine activity by reducing the triggering threshold only in genetically predisposed individuals.[66]

$Ca_v2·1$ 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.[134,135] Stimulation of isolated trigeminal ganglia led to higher neuronal release of CGRP in Arg192Gln mice than was noted in wild-type mice.[136] The increased CGRP concentrations mainly affected neighbouring satellite glial cells, creating a local persistent inflammatory environment and peripheral sensitisation.[136–138] This effect was evidenced by upregulation of purinergic signalling via neuronal P2X3[139] and satellite glial cell P2Y receptors,[136] larger basal release of TNFα,[120] and greater activation of macrophages.[119,120] Finally, trigeminovascular responses to nociceptive electrical stimulation of the superior

	Patients with familial hemiplegic migraine	Arg192Gln and Ser218Leu FHM1 mouse models
Transient motor weakness	Transient motor weakness during migraine attacks[65]	Episodes of transient motor weakness, occurring either spontaneously (Ser218Leu) or after experimental induction of cortical spreading depression (Arg192Gln and Ser218Leu)[105]
Headache and associated features during attack	Headache, photophobia, and other associated symptoms[65]	Photophobia and head pain, disappearing after treatment with rizatriptan[107,108]
Cerebellar signs	Up to 20% of patients with FHM have cerebellar ataxia;[65] subclinical signs of cerebellar ataxia have been shown in patients with migraine[111]	Cerebellar ataxia in Ser218Leu mice, improving after activation of Ca^{2+}-dependent K^+ channels[110]
Cerebellar atrophy	Up to 25% of patients with FHM have cerebellar atrophy[65]	Ser218Leu mice develop Purkinje cell abnormalities[83]
Seizures	Seizures may occur in patients with FHM, either independently or as part of severe attacks[65]	Seizures may occur in Ser218Leu mice, either spontaneously or after experimental induction of cortical spreading depression[83-105,106]
Severe attacks with cerebral brain oedema	Severe attacks might occur with decreased consciousness, agitation, confusion and seizures in up to a third of patients with FHM. Fatal episodes of deep coma, severe cerebral oedema, and seizures might occur after mild head trauma in *CACNA1A* mutation carriers[65]	Coma and often fatal seizures might occue in Ser218Leu mice, mainly after experimental induction of cortical spreading depression, but sometimes also spontaneously[83,105-106]
Female preponderance and influence of female hormones	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)[65]	Susceptibility to cortical spreading depression, as a surrogate marker for migraine susceptibility, is higher in female FHM1 mice and can be modulated by experimentally manipulating female hormone levels[105,112]
Mutation and phenotype severity-correlated changes	Depending on the specific mutation, patients may only have episodes of hemiplegic migraine or also additional ictal or permanent features such as cerebellar ataxia and atrophy, seizures, and fatal brain oedema[65]	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[82,83,105-107,112,113]

FHM=familial hemiplegic migraine.

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

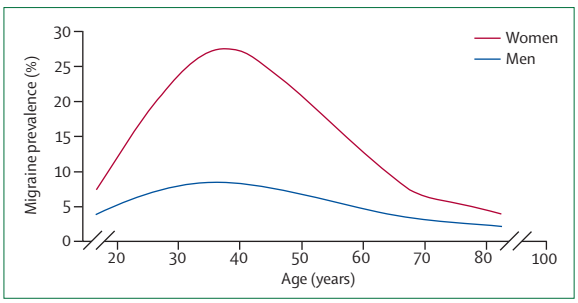

Figure 5: **1 year prevalence of migraine in the general population**
Adapted from Lipton and colleagues,[130] Payne and colleagues, [131] and Peterlin and colleagues.[132] 1 year prevalence of migraine is defined as the proportion of subjects from the general population who have been diagnosed with 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.

sagittal sinus were lower in FHM1 than in wild-type mice, but the dependence on activation of trigeminothalamic neurons was higher.[121]

Mechanisms for migraine comorbid diseases

Migraine with aura is associated with increased risk of ischaemic stroke, particularly in women;[37,140] migraine might even be a greater risk factor for stroke than hypertension and diabetes.[141] Studies in FHM1 mice have suggested that neuroexcitatory mechanisms implicated in migraine might also increase vulnerability to ischaemic injury.[142] 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.[142] 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 $Ca_v2·1$ channel dysfunction and worse clinical phenotype in these mice).[82,83] Treatment with the NMDA glutamate receptor antagonist MK-801 reduced infarct size and improved outcome, implicating migraine-related mechanisms as potential targets for prevention and treatment of stroke.[142] In line with these observations, Leaner mice, who are pathogenetically the opposite of FHM1 mice (loss-of-function instead of gain-of-function mutations in the *Cacna1a* gene)[127] showed smaller infarct sizes than controls.[143]

Several lines of research also point at $Ca_v2·1$ channel dysfunction as a possible explanation for the comorbidity of migraine and FHM with epilepsy and cerebellar ataxia.[31,54,65,91] 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.[83] The ataxia most likely is caused by irregular firing of Purkinje cells, due to mutant $Ca_v2·1$ channels lowering the threshold for somatic action potentials and dendritic Ca^{2+} spikes.[113] Activators of Ca^{2+} dependent K^+ channels improved motor performance by normalising Purkinje cell function.[113]

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.[144] Mutant protein concentration in the brain was strongly decreased in heterozygous mutant mice and almost undetectable in homozygous mutants (which died immediately after birth).[144] 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.[144] 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 $Ca_v2·1$ *CACNA1A* gain-of-function mutations cause increased neuronal release of glutamate[82–84] and FHM2 Na^+/K^+-ATPase *ATP1A2* loss-of-function mutations cause reduced glial cell reuptake of glutamate from the synaptic cleft.[144] FHM3 $Na_v1·1$ *SCN1A* loss-of-function mutations, although not yet studied in vivo, are predicted to cause increased activity of glutamatergic excitatory neurons via decreased GABA-ergic interneuronal inhibition.[88]

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.[145] Because CADASIL *NOTCH3* mutations mainly affect blood vessels,[67] 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 allodynia, increased neuronal activation within the trigeminal nucleus, female preponderance, increased calcium signalling in astrocyte cultures, and disruption of circadian rhythms.[64] Most importantly, the cortical spreading depression triggering threshold was decreased.[64] Finally, a transgenic knock-in mouse model for CHARIOT was generated in which the human pathogenic truncating *TREX1* V235fs (frameshift) mutation[61] was introduced into the orthologous *Trex1* mouse gene by use of a gene-targeting approach.[146] 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.[90]

Although no evidence supports the idea that FHM or other genes regulating ion-homoeostasis are directly involved in migraine,[147] 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*,[63] the EAAT1 glutamate transporter gene *SLC1A3*,[100] and the Na^+–$HCO3^-$ co-transporter NBCe1B/C pH regulating gene *SLC4A4*[104] in glial cells.

Functional tests have suggested subclinically impaired cerebellar coordination in migraineurs[148] and single-fibre studies in migraineurs,[149] but not in patients with FHM1,[150] were consistent with altered release of acetylcholine at the neuromuscular junction. As P/Q-type $Ca_v2\cdot1$ 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.[151] 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,[152] particularly migraine with aura.[153] Transcranial magnetic stimulation during aura, possibly blocking cortical spreading depression, prevented progression from aura to headache more frequently than did sham stimulation.[154] Several,[155,156] but not all,[111] 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),[157,158] although a third study[159] 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 modulating 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.[160]

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.

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.

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 $Ca_v2·1$ Ca^{2+} channel activity, normalising the cellular effects of FHM1 mutations.[161] Results from such studies might not only benefit patients with migraine but also patients with pathophysiologically related and frequently comorbid brain disorders such as epilepsy, depression, and cerebellar ataxia.

Contributors

MDF and RRK contributed equally to the design and literature search of most of the Review with help from GMT, AMJMvdM and CA for expertise areas. All authors contributed to the writing of the review.

Declaration of interests

The authors declare no competing interests.

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