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Animal models of monogenic migraine

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Abstract

Migraine is a highly prevalent and disabling neurological disorder with a strong genetic component. Rare monogenic forms of migraine, or syndromes in which migraine frequently occurs, help scientists to unravel pathogenetic mechanisms of migraine and its comorbidities. Transgenic mouse models for rare monogenic mutations causing familial hemiplegic migraine (FHM), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and familial advanced sleep-phase syndrome (FASPS), have been created. Here, we review the current state of research using these mutant mice. We also discuss how currently available experimental approaches, including epigenetic studies, biomolecular analysis and optogenetic technologies, can be used for characterization of migraine genes to further unravel the functional and molecular pathways involved in migraine.

Keywords

Genetic animal model, migraine without aura, pathophysiology

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The lack of neurobiological knowledge about migraine and its comorbidities

Migraine is a neurological disorder characterized by recurrent debilitating attacks of headache that are typically associated with nausea, vomiting, and hypersensitivity to light, sound, and smell (1). It is ranked the third-most common disease worldwide, and second-most common neurological disorder that accounts for more than half of the number of years lived with disability attributable to neurological diseases (2). Migraine is divided into two major categories, migraine without aura (MO) and migraine with aura (MA) (1), the latter affecting up to one-third of patients. Aura symptoms are transient neurological symptoms including visual, sensory, motor or speech disturbances, which gradually develop most of the time prior to headache onset, with a typical duration of 5–60 minutes. Migraine is comorbid with a variety of neurological or psychiatric disorders (3–5), such as ischemic or hemorrhagic stroke (6–9), epilepsy (10,11), restless legs syndrome (12,13), depression (14,15), and panic (16) or anxiety disorders (17). Despite comprehensive characterization of clinical features, detailed molecular and neurobiological knowledge of migraine as well as its association with comorbidities is still lacking.

Animal studies have helped to better understand migraine pathophysiology and to identify potential therapeutic targets (18–22). Migraineurs' brains seem more vulnerable to perturbations of ion homeostasis and environmental changes that might trigger attacks by activation and sensitization of the trigeminovascular system (TVS) (23–26). Experimental and clinical data suggest a pivotal role for cortical spreading depression (CSD) as part of headache initiation mechanisms (23–26), although it should be emphasized that this is debated for migraine attacks not preceded by aura (27,28). CSD represents a wave of neuronal and glial depolarization that spreads with a velocity of 3–6 mm/min across the cerebral cortex, and is considered the electrophysiological substrate of migraine

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aura (29). Upon CSD, inflammatory cascades are initiated, reportedly associated with the opening of neuronal Pannexin1 mega channels that causes release of inflammatory mediators including nuclear factor (NF)κB, and subsequent trigeminovascular activation (26). In animal models, CSD evokes a cascade of cortical, meningeal and brainstem events consistent with the development of headache (23,26). Subcortical structures such as diencephalic and brainstem nuclei are affected by CSD as well (30) and might contribute to the pathophysiology of migraine by modulating the perception of TVS activation. The connections of these diencephalic and brainstem nuclei to other brain regions could also contribute to migraine-associated symptoms or comorbidities, such as photophobia or restless legs syndrome (21,31).

Accumulating evidence supports an important role for genetic factors in determining migraine susceptibility (32,33). Nevertheless, migraine is considered a complex disorder for which only a few monogenic causes have been identified (34). Given the overlap in clinical features with common migraine, and the availability of mutant mouse models, monogenic forms of migraine serve as useful models for in-depth exploration of migraine biology. In this review, we provide a comprehensive overview of the current knowledge and future directions of research using genetic animal models for monogenic forms of migraine (see Figure 1) and discuss their limitations in the context of common forms of migraine.

Monogenic migraine as a model for disease

Studying effects of mutations associated with rare monogenic forms of migraine provide insight into genes and pathways involved in migraine susceptibility. Pathogenic mutations causing familial hemiplegic migraine (FHM) type 1 and 2, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and familial advanced sleep-phase syndrome (FASPS) formed the basis for the currently available transgenic mouse models for monogenic disorders associated with migraine. As outlined below, enhanced CSD susceptibility has been identified in these transgenic mouse models despite different underlying mutations and affected pathways. Enhanced cortical glutamatergic transmission may underlie the increased CSD susceptibility, which has been shown for FHM1 mouse models (35). Indirect evidence emphasizing the importance for neuronal excitability in determining CSD susceptibility comes from experiments showing that antiepileptic drugs that suppress neuronal excitability (36,37) also reduce CSD susceptibility (38). In addition, clinical studies in patients with FHM1 (39) and common forms of migraine (40,41) as well as findings from genome-wide association studies (GWAS) in patients with common migraine (18) support the concept of cortical hyperexcitability underlying, at least to some extent, migraine. It is likely that additional mouse models will be generated, based on other mutations associated with the above-mentioned diseases as well as other monogenic forms of migraine. To specifically distinguish between mechanisms related to monogenic forms of migraine with or without aura, novel animal models have been developed. Worth mentioning in this context are three prime repair exonuclease 1 (*TREX1*) mutations implicated in endothelial function and associated with migraine mainly without aura (42,43). Recent animal studies involving altered calcitonin gene-related peptide (CGRP) receptor pathways represent promising additional genetic and molecular models of migraine headache (44,45).

FHM

FHM is a rare subtype of monogenic MA that is characterized by transient weakness (hemiparesis) during the aura phase of some attacks (1). Apart from hemiparesis, the headache and aura features of FHM attacks are identical to those of common migraine attacks (46). In addition to hemiplegia, the majority of FHM patients also experiences attacks of "normal" migraine with or without aura (47). Thus, from a clinical point of view, FHM seems part of the migraine spectrum, and a valid model to study common forms of migraine. So far, three FHM genes have been identified: calcium channel, voltage dependent, P/Q type, alpha 1A subunit (*CACNA1A*) (FHM1); ATPase, Na^+/K^+ transporting, alpha 2 polypeptide (*ATP1A2*) (FHM2); and sodium channel, voltage gated, type 1 alpha subunit (*SCN1A*) (FHM3), which encode subunits of voltage-gated calcium channels, sodium-potassium ATPases, and voltage-gated sodium channels, respectively.

The first FHM gene (FHM1), *CACNA1A*, is located on chromosome 19p13 and codes for a subunit of neuronal voltage-gated $Ca_V2.1$ Ca^{2+} channels (48). These channels modulate release of neurotransmitters at most central synapses throughout the brain (49). Biophysical analysis of FHM1 mutations shows gain-of-function of human $Ca_V2.1$ channels expressed in heterologous cell systems (50) as well as gain-of-function of neuronal $Ca_V2.1$ channels in FHM1 KI mice (51). The gain-of-function effect was shown to increase action-potential evoked Ca^{2+} influx and enhance probability of glutamate release in the synaptic cleft (35,52). During enhanced neuronal network activity, this would facilitate CSD (53) and thereby could explain migraine

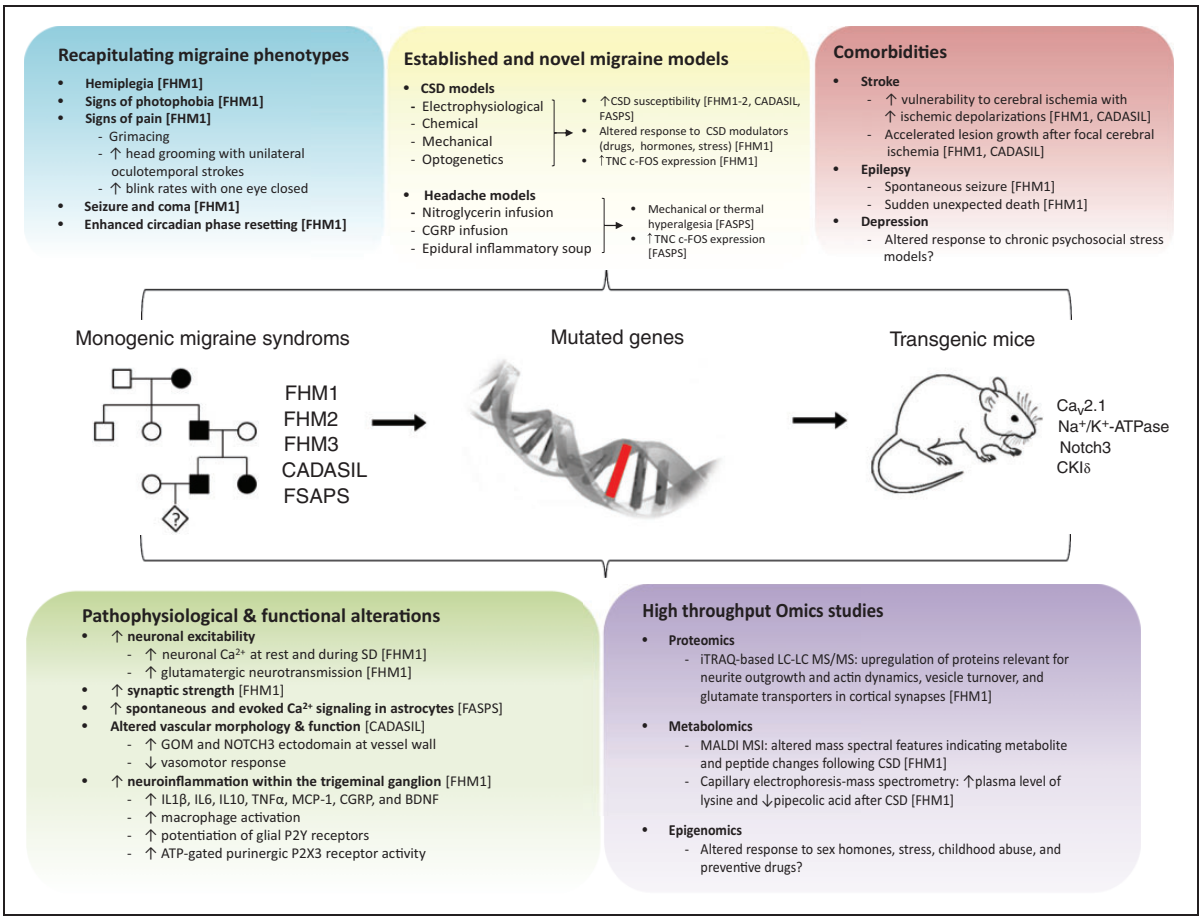

Figure 1. Current and future directions for research using animal models of monogenic migraine.
Monogenic mutations causing familial hemiplegic migraine (FHM), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukencephalopathy (CADASIL), and familial advanced sleep-phase syndrome (FASPS), have been identified and corresponding transgenic mice have been created (middle panel). These transgenic mice recapitulate migraine phenotypes in patients (upper left box) and have been proven useful to study migraine pathogenesis. For example, these animal models have been employed in established and novel models for migraine aura and headache using standardized experimental protocols, and universally accepted migraine-relevant readouts (upper middle box). Furthermore, these migraine animal models have also been used to study comorbidities of migraine, such as stroke and epilepsy, and are expected to also be suitable for studying other comorbidities such as major depression (upper right box). Recent in vitro and in vivo studies have demonstrated functional alterations in these mice (lower left box), which helped to better understand migraine pathophysiology. Cutting-edge technologies for analysis of systems biology changes are being implemented at the level of proteomics, metabolomics and epigenetics, to further characterize the functional and molecular pathways involved in migraine pathogenesis (right lower box).

aura symptoms (and consequently headache) associated with FHM1. Clinical variability associated with FHM1 mutations ranges from pure hemiplegic episodes, as observed for carriers of the R192Q mutation (48), to FHM with severe neurological deficits including cerebellar ataxia, epilepsy, or even fatal coma, as seen in patients carrying the S218L mutation (54) (for a review, see de Vries et al. (55)).

The second FHM gene (FHM2), *ATP1A2*, is located on chromosome 1q23 and encodes the α2 subunit of the Na^+/K^+ pump ATPase (56). This catalytic subunit binds Na^+, K^+, and ATP, and uses adenosine triphosphate (ATP) hydrolysis to move Na^+ ions out of the glial cell in exchange for K^+ ions. Thus, the ATPase can modulate re-uptake of potassium and glutamate from the synaptic cleft into glial cells. At the cellular level, FHM2 mutations cause a loss of function of the glial Na^+/K^+ ATPase, which is hypothesized to increase extracellular levels of glutamate and K^+, and consequently, could indirectly cause neuronal hyperexcitability (57,58). However, the relative roles of the three different cerebral Na^+/K^+ ATPases in K^+ and glutamate clearance still need to be characterized (59,60). FHM2 mutations have been associated with

pure hemiplegic symptoms in association with FHM attacks (56,61,62) or a combination of FHM symptoms with other neurological deficits, such as cerebellar ataxia, recurrent coma, aphasia, impaired hearing and behavioral changes (63–65). In some cases, migraine co-occurred with childhood epilepsy, which could be linked to novel missense mutations in the *ATP1A2* gene (66).

The third FHM gene (FHM3), *SCN1A*, is located on chromosome 2q24 and encodes a subunit of voltage-gated $Na_V1.1$ sodium channels (67) that play an important role in the generation and propagation of action potentials. Apart from migraine and hemiplegia, FHM3 mutations are sometimes associated with additional clinical features such as childhood epilepsy, generalized tonic-clonic epilepsy and transient blindness (67–70). For the described FHM3 cases in which FHM co-occurred with epilepsy, seizures were observed independently from hemiplegic migraine attacks (68), suggesting shared molecular pathways between FHM and epilepsy.

FHM3 seems to be associated with gain-of-function of $Na_V1.1$ channels and consequent hyperexcitability of, most likely, inhibitory interneurons. However, conflicting findings on the effect of FHM3 mutations on recombinant $Na_V1.1$ channels have been reported, pointing to either gain- or loss-of-function effects depending on the mutation and/or the study. Taken together, these findings indicate that the spectrum of $Na_V1.1$ defects causing FHM3 is complex. FHM3 gain-of-function effects appear in contrast with the observed loss-of-function mutations in $Na_V1.1$ channels in the context of epilepsy that are linked with impaired firing of inhibitory interneurons (71). Functional studies in brain slices or in vivo are needed to reveal the neuronal network mechanisms by which FHM3 mutations, with either loss- or gain-of-function effects, contribute to co-occurrence of epilepsy and migraine in patients (72,73).

Other monogenic disorders associated with migraine: CADASIL and FASPS

Other rare monogenic syndromes in which migraine frequently occurs provide useful additional insight in pathophysiological mechanisms.

A prominent example is CADASIL, one of the most common hereditary cerebral small vessel diseases. CADASIL is attributed to mutations in the *NOTCH3* gene, a highly conserved gene located on human chromosome 19q13, encoding a transmembrane receptor expressed in smooth muscle cells and pericytes of small vessels (74). CADASIL is a cerebral microangiopathy characterized by several pathological and clinical hallmarks. Typical pathological findings of CADASIL include accumulation and deposition of the extracellular domain of NOTCH3 and granular osmiophilic material (GOM) in the vascular wall of small-caliber arteries and arterioles, resulting in gradual degeneration of the vascular smooth muscle cells, adventitial fibrosis, mural thickening and luminal narrowing (75–77). Although the brain is the predominant manifestation site, microvascular changes also occur systemically. In fact, aggregates of the NOTCH3 extracellular domain and GOM deposits can be detected in skin vessels more than a decade prior to clinical manifestation of the disease (78–81). Around 30%–40% of patients with CADASIL have migraine attacks (82), with a high prevalence in Caucasian (14% to 77%) (83–85) but low prevalence in Asian patients (3% to 5%) (86,87). CADASIL in many cases first clinically manifests with migraine, often with characteristic visual or sensory aura. About half of the patients have atypical presentations of migraine attacks, such as aura without headache, hemiplegic or brainstem aura, prolonged aura or acute-onset aura (88). Some patients might develop confusion, fever, meningitis, or coma (88–90). Later on, recurrent ischemic strokes, psychiatric disturbances, and finally subcortical ischemic vascular dementia dominate the clinical picture (83,88,91,92). A possible role of the *NOTCH3* gene in modulating susceptibility to migraine in the general population is suggested by a recent study demonstrating an association of the G684A (rs1043994) variant in exon 4 of the *NOTCH3* gene with migraine, specifically for patients with MA (93). However, other studies have shown that although the prevalence of migraine in CADASIL families is high, *NOTCH3* genotypes might not be the only factor contributing to migraine phenotypes (94,95). Some other genetic or environmental factors might also play a role in increasing susceptibility to migraine in family members of CADASIL patients (83,85).

Another monogenic disorder associated with MA is the FASPS. FASPS is an autosomal dominant disorder characterized by a shift of the circadian cycle, presenting as early evening sleep onset and early morning awakening. A causal missense mutation has been identified in the *CSNK1D* gene, which encodes casein kinase Iδ (CK1δ) (96). CK1δ is a member of the Casein kinase 1 (CK1) family serine/threonine kinases, which regulate multiple cellular processes by phosphorylating proteins involved in cell differentiation, proliferation, chromosome segregation and circadian rhythms (97,98). CKIδ is known to regulate the temporal abundance and activity of the circadian clock protein Per2, via phosphorylation-mediated degradation and cellular localization (99). Mice and flies carrying the same missense mutation in human T44A in the *CSNK1D* gene show significant alteration of circadian cycles (96). In two

independent families with FASPS, two distinct missense mutations (T44A and H46R) identified in the *CKIδ* gene were found to co-segregate with MA (100). The associations between the mutations and clinical phenotypes suggest that the mutant gene product of CK1D contributes to the pathogenesis of migraine.

Mouse models of FHM

Two transgenic FHM1 knock-in (KI) mouse models have been generated, expressing the gain-of-function missense mutations R192Q or S218L in the *Cacna1a* gene (51,101). Mutant mice with the milder R192Q mutation do not display an overt phenotype (51), while mice carrying the severe S218L mutation exhibit a complex phenotype including cerebellar ataxia and spontaneous seizures (101), in accordance with the clinical phenotype in FHM1 S218L patients. As outlined in detail below, functional studies of FHM1 KI mice suggest alterations in excitation/inhibition balance (35,102,103), synaptic plasticity (104) and CGRP-mediated trigeminal pain signaling (105–107) in addition to enhanced susceptibility to experimentally induced CSD (51,101,108–110), as part of mechanisms underlying migraine. FHM2 KI mice were generated that express the loss-of-function W887R missense mutation in the *Atp1a2* gene. Heterozygous FHM2 KI mice display an increased CSD susceptibility in vivo (111), as seen with FHM1 KI mice. Similar to homozygous *Atp1a2* knock-out mice (112), homozygous FHM2 KI mice are not viable, leaving only heterozygous mice for studies. Thus far, no FHM3 KI mouse model has been generated.

FHM1 mice display $Ca_V2.1$ gain of function and enhanced CSD susceptibility

FHM1 mutations cause a $Ca_V2.1$ gain of function, potentially underlying the cerebral hyperexcitability observed in FHM1 patients (39).

FHM1 R192Q (35,102) and S218L (103) mutations were shown to enhance excitatory but not inhibitory neurotransmitter release in cortical brain slices and neuronal cultures. Enhanced cortical glutamatergic synaptic transmission was associated with increased CSD susceptibility, as shown for R192Q KI mice in cortical brain slices in vitro (35). When cortical excitatory transmission was reduced to wild-type (WT) level by partially inhibiting Ca^{2+} channels, the facilitation of CSD susceptibility was normalized (35), thus providing a causative link between enhanced Ca^{2+} influx, glutamatergic synaptic transmission and susceptibility to CSD. A recent in vivo Ca^{2+} imaging study in the somatosensory cortex of FHM1 S218L KI mice showed elevated neuronal Ca^{2+} levels at rest, associated with an altered synaptic morphology (113) that is compatible with stronger synapses and a hyperexcitability phenotype. These findings suggest that S218L mutant channels are facilitated at rest. The observation of an increased frequency of miniature excitatory postsynaptic currents (mEPSCs) in cortical slices in S218L (103) but not R192Q KI mice (35) may suggest that increased resting Ca^{2+} levels could be characteristic for S218L KI mice, contributing to S218L-specific neurological symptoms such as epilepsy. In vivo under anesthesia, CSD susceptibility was also increased (5,101,108–110), more prominent in S218L compared to R192Q KI mutants, and further modulated by sex and stress hormones (109,114). The enhanced in vivo CSD susceptibility of homozygous compared to heterozygous S218L mice (101,108) was recently proposed to be related to a fraction of $Ca_V2.1$ channels in S218L homozygous KI cortical synapses already being open at membrane potentials below the threshold for action potential generation (103). These observations are in line with the more severe neurological deficits in patients carrying the S218L mutation compared to the R192Q mutation.

The effect of FHM1 mutations on synaptic plasticity depends on the neuronal network

At the cortical level, in vitro studies demonstrate an enhancement of synaptic depression for R192Q (35) and S218L KI mice (103), suggesting a decreased efficacy of synaptic transmission during repetitive stimulation. In vitro and in vivo findings of the brainstem Calyx of Held synapse of homozygous S218L KI mice showed a faster rate of recovery from synaptic depression compared to WT (115) that was also observed in vitro for R192Q KI mice (116). In the cerebellum, a reduction of short-term synaptic facilitation at the parallel fiber-to-Purkinje cell synapses in slices from R192Q and S218L KI mice could be related to a facilitated state of mutant presynaptic Cav2.1 channels (52) that impair the balanced tuning of Purkinje cell firing, thereby contributing to cerebellar ataxia associated with FHM1. A recent in vivo study on hippocampal function in R192Q and S218L KI mice showed that enhanced long-term potentiation with unaltered long-term depression were related to impaired learning and memory behavior, possibly resulting from an imbalance between potentiation and depression at the neuronal circuit level (104). It remains to be seen how neuronal network changes in the brainstem, hippocampus and cerebellum translate to functional FHM1 characteristics of migraine-relevant neuronal networks.

As evident from different effects of the same mutation in cortex compared to the trigeminal ganglion (TG) (106) and brainstem (117), functional effects of

FHM1 mutations can be neuron type specific. For example, the absence of an effect of FHM1 mutations on cortical fast-spiking interneurons in comparison to a clear gain-of-function effect observed for excitatory pyramidal neurons appears due to the expression of interneuron-specific $Ca_V2.1$ channels whose gating properties are not modified by the FHM1 mutation (102). Functional differences between types of neurons as shown for healthy cortical networks (118) may explain distinct effects of FHM1 mutations on different neuronal networks that could be hypothesized to result in dynamic disturbances in the balance between excitation and inhibition in specific neuronal circuits.

Altered trigeminovascular processing and a molecular pain phenotype in FHM1 mice

$Ca_V2.1$ channels are known to facilitate release of glutamate and CGRP from trigeminal neurons in vitro (119) as well as nociceptive transmission in the trigeminocervical complex (TCC) in vivo (120). Studies investigating TG function of FHM1 mice revealed a molecular pain phenotype involving altered purinergic signaling by CGRP. TG sensory neurons of R192Q KI mice displayed enhanced ATP-gated purinergic P2X3 receptor activity as a result of an altered phosphorylation state of intracellular protein domains (121). The constitutive activation of P2X3 receptors is likely related to the enhanced Ca^{2+} influx in FHM1 mutant neurons since P2X3 membrane expression was unaltered. Furthermore, TG neurons of R192Q KI mice exhibited increased release of various soluble mediators such as CGRP, brain-derived neurotrophic factor (BDNF) and tumor necrosis factor alpha (TNFα) already at baseline that may contribute to the increased P2X3 receptor activity and could explain why further potentiation of these receptors by exogenous CGRP or TNFα was not observed (122). The enhanced purinergic activity was linked with abnormal cytokine/chemokine profiles and the presence of activated macrophages as observed for TG of FHM1 R192Q KI mice (123). CGRP release was reportedly increased in the TG while it was unaltered in the dura of R192Q KI mice, consistent with an observed lack of effect of the FHM1 mutation on $Ca_V2.1$ currents in capsaicin-sensitive TG neurons that constitute a majority of small dural afferents (106). When FHM1 TG neurons were co-cultured with satellite glial cells, an increased CGRP release at baseline and upon neuronal activation resulted in potentiation of glial P2Y receptors and subsequent glial activation (107). This may seem in contrast with the observed reduction of CGRP-immunoreactive neurons (with unaltered total number of neurons) in TG and superficial laminae of the TCC in R192Q KI mice and could reflect reduced CGRP synthesis (105). Alternatively, however, reduced CGRP expression may reflect depletion of intracellular CGRP stores in the FHM1 mutant mice as a result of an overactive TVS with enhanced CGRP release. Taken together, the neuroinflammatory state of TGs in FHM1 KI mice, with constitutively activated purinergic receptors both in neurons and glial cells, could facilitate pain signal transduction given the role of inflammatory pain mediators acting on meningeal nociceptors in the context of head pain development (124). Regarding alterations in other parts of the TVS in FHM1 mice, upon dural activation, the number of cFos expressing cells in the TCC was reduced in R192Q KI mice compared to WT, whereas the number of cFos expressing cells in medial and posterior trigeminothalamic nuclei was enhanced (125). These differential changes in trigeminovascular nociceptive pathway activation in FHM1 mice may suggest that the enhanced opening probability of mutant $Ca_V2.1$ channels has more pronounced effects on higher-order neurons (at the level of the thalamus) compared to lower-order neurons (at the level of TCC).

Behavioral signs of pain in FHM1 mice and possible association with a molecular pain phenotype

Awake FHM1 R192Q and S218L mutant mice exhibit behavioral changes suggestive of spontaneous unilateral head pain. Behavioral signs of pain including increased amount of head grooming with unilateral oculotemporal strokes and increased blink rates with one eye closed, appeared novelty and/or restraint stress induced. For mutants carrying the severe S218L mutation, eye blink behavior was enhanced compared to R192Q mice. This likely pain-related behavior could be normalized by serotonergic antimigraine drugs, suggesting involvement of trigeminal pain pathways (126). Further evidence for spontaneous head pain in FHM1 mice has come from assessment of the so-called mouse grimace scale, an objective measure of facial pain expression in mice. Grimacing was enhanced in R192Q KI mice and could be normalized by antimigraine drugs (127). In addition to potential signs of head pain, FHM1 mice displayed signs of photophobia by exhibiting light-avoidance behavior (126).

If the in vitro findings from CGRP changes in relation to TG function (see Altered trigeminovascular processing and a molecular pain phenotype in FHM1 mice) translate to the in vivo level, FHM1 mice could serve as a valuable model for investigating effects of existing and novel migraine drugs, which may involve modulation of CGRP and inflammatory pathways (128,129). Comparison of behavioral changes in FHM1 mice to photophobic behavior observed in a transgenic mouse

model sensitized to CGRP (44,45) would provide useful additional insights regarding mechanisms of pain behavior.

FHM2 KI mice show reduced astrocytic α2 Na^+,K^+-ATPase expression and enhanced susceptibility to CSD

The α2 Na^+,K^+ ATPase affected by FHM2 mutations (56,130) is an ATPase isoform that in the adult brain is predominantly expressed in astrocytes (131,132). Although direct evidence has as of yet not been provided, the α2 Na^+, K^+ ATPase is expected to play a strong role in buffering excessive extra-synaptic K^+ during neuronal activation (59,133). Heterozygous FHM2 *Atp1a2*$^{+/R887}$ mice exhibit markedly reduced α2 Na^+,K^+-ATPase protein expression in the brain that is probably attributed to inefficient and delayed secretion by the endoplasmic reticulum and impaired degradation by proteasomes (111). FHM2 *Atp1a2*$^{+/R887}$ mice display no apparent clinical phenotype except for higher fear and anxiety scores in the SHIRPA primary screening. Similar to FHM1 mice, heterozygous FHM2 mice display a decreased threshold for electrically induced CSD with increased CSD propagation speed (111). Likely, the enhanced CSD susceptibility in these mice is due to impaired astrocytic K^+ and glutamate clearance and related increase in cortical excitatory neurotransmission, although this remains to be proven.

Mouse models of CADASIL

Most of the pathogenic mutations in CADASIL patients identified so far represent mutations affecting the NOTCH3 extracellular domain (134–137). These mutations result in unique protein-protein interactions of mutated protein, although whether the effect is mediated by loss or gain of function is still in debate (136–138). *Notch3* knockout mice exhibit ultrastructural and functional abnormalities of vascular smooth muscle cells, but do not harbor parenchymal pathology resembling CADASIL (139–142), which may be due to the fact that strokes and small-vessel disease occur during late stages of CADASIL and the life span of a mouse is short. However, several *Notch3* mutant mouse models have been developed using KI and transgenic approaches (143–146), with some of them showing pathological hallmarks of CADASIL. These mice develop Notch3 extracellular domain aggregates and GOM deposits, resulting in CADASIL-like vasculopathy and white matter disease features, especially in the mutant strain that employs the Notch3 promoter (TgPAC-*Notch3*^{R169C}). However, the phenotypic spectrum of these experimental models is heterogeneous and the expression level of Notch3 varies, possibly due to the difference of genetic constructs and mouse lines (145,147).

Despite the aforementioned phenotypic heterogeneity, these *Notch3* mutant mouse models have provided opportunities to explore the molecular and physiological mechanisms of CADASIL as well as migraine. CSD has been investigated in a transgenic KI mouse model of CADASIL carrying the R90C mutation as well as in *Notch3* knockout mice. Both R90C transgenic (neomorphic) and *Notch3* knockout mice exhibit a reduced threshold for CSD induction, increased CSD frequency upon continuous topical application of KCl and an increased CSD propagation speed (148), providing a possible explanation for the high incidence of MA in CADASIL patients with either gain- or loss-of-function *NOTCH3* mutations. The exact mechanism of increased CSD susceptibility remains to be explored, but abnormal NOTCH3 receptor signaling and microcirculatory dysfunction (resulting in transient hypoperfusion, impaired homeostasis of ions and energy, or even ischemic lesions) have been implicated (148).

Mouse model of FASPS

Transgenic mice expressing the *CKIδ-T44A* allele have been found to exhibit an increased CSD susceptibility with a reduced CSD threshold and increased frequency of KCl-evoked CSDs (100). This phenotype seems to be more modest in comparison with transgenic mouse models of FHM and CADASIL (51,108,111,148), although head-to-head comparisons have not been employed and experimental protocols were different. Importantly, *CKIδ-T44A* transgenic mice exhibit additional migraine-relevant phenotypes that have not been tested in the other mutant mouse strains yet, such as nitroglycerin (NTG)-induced mechanical and thermal hyperalgesia as well as cFos-activation in the trigeminal nucleus caudalis (TNC). Furthermore, these mutants also displayed increased spontaneous and evoked calcium signaling in astrocytes and enhanced CSD-evoked pial arterial dilation. Further work is required to dissect underlying mechanisms by which a single *CKIδ-T44A* gene mutation can produce such diverse phenotypes.

How relevant are the mouse models and what have they taught us about migraine?

FHM1 mice represent the best-studied genetic monogenic migraine mouse model, for which several migraine-associated features have been described including spontaneous behavior indicative of pain and photophobia (see Behavioral signs of pain in FHM1

mice and possible association with a molecular pain phenotype). Several of the functional readouts in FHM1 mice are affected by allele dosage, and, most important, by the type of FHM1 mutation: Strongest effects are observed for S218L homozygous in comparison to S218L heterozygous and R192Q homozygous KI mice (101–104,108,126). Interestingly, the CSD-induced transient neurological deficits in FHM1 mice resemble migraine aura symptoms of patients with the respective mutations, with R192Q mice developing pure hemiparesis, and S218L mice exhibiting additional neurological symptoms including seizures upon experimentally induced CSD (108). Of note, some of these behaviors normalized after administration of the antimigraine drug rizatriptan (126,127), supporting the validity of these models for studying migraine pathophysiology. FHM mutant mice show an altered response to migraine-relevant triggers, such as an increased CSD susceptibility secondary to rises in stress hormones (114) and an enhanced circadian phase resetting after experimental jet lag (149). In addition, the increased CSD susceptibility in FHM1 transgenic mice is reduced by male and further enhanced by female sex hormones (108), consistent with the female preponderance in migraine (150,151). Similarly, female *CKIδ-T44A* transgenic mice also exhibit a reduced threshold of NTG-induced thermal hyperalgesia in comparison with male or WT mice (100). Even though mutation-specific migraine features in mouse models predict high relevance of study results for patients with the same mutation, careful comparison with other mouse models is necessary before drawing conclusions for more common forms of migraine. Many of the CSD features tested in S218L mice have been reproduced in R192Q carriers, such as the effect of age, gender or migraine-prophylactic drugs, but severe neurological symptoms present only in S218L, warranting caution not to over-interpret results gained from S218L mice before confirming findings in other animal models. Therefore, future work should test migraine-relevant features across different monogenic mouse models, and include models more specific for migraine headache such as CGRP models (44,45) or mice carrying *TREX* mutations (42,43,152).

Functional analysis at the cellular and network level in the R192Q FHM1 mouse model revealed increased excitatory glutamatergic neurotransmission to underlie enhanced CSD susceptibility (35). Increased neuronal calcium levels and an abnormal synaptic morphology were proposed to contribute to hyperexcitability, based on findings in cortical neurons of FHM1 S218L KI mice (113). Whether these observations relate to migraine pathophysiology, or rather to other neurological features like epilepsy that are specific to S218L mice, remains to be explored. Furthermore it remains to be determined whether cerebral hyperexcitability is also a feature of the FHM2, FASPS, and CADASIL mouse models. As noted above, effects of FHM1 mutations can differ among neurons of specific brain regions (106,117) and, as shown for the cortex, appear more pronounced for excitatory than for inhibitory neurons (35,102,103). The observed changes in inflammatory cascades and CGRP modulation in the TG of FHM1 mice (see Altered trigeminovascular processing and a molecular pain phenotype in FHM1 mice), in particular if confirmed at other levels of the TVS, make FHM1 mice well suited to investigate mechanisms linking hyperexcitability, CSD, inflammation and headache (26,128).

In addition to neuronal excitability, cerebral vasculature with its coupling to neuronal function appears also important for migraine pathogenesis (42,153,154). Since the major manifestation site of CADASIL mutations is at the level of (small) cerebral blood vessels, it is reasonable to implicate vascular mechanisms for the occurrence of migraine attacks in CADASIL patients. As mentioned in Mouse models of CADASIL, microcirculatory dysfunction is considered the culprit of the increased CSD susceptibility in *Notch3* mutant mice. However, such a relationship may be specific to CADASIL since the prevalence of MA does not seem to be remarkably increased in patients with chronic hypertension-related small-vessel diseases (92). Consistent with this notion, animals with bilateral common carotid artery stenosis-related chronic forebrain hypoperfusion do not show enhanced CSD susceptibility (148). In addition, CSD-induced blood flow changes in CADASIL mutants did not differ from that of WT mice (148). Hence, factors other than cerebral hypoperfusion, such as platelet dysfunction, inflammation, or microemboli that could contribute both to microcirculatory dysfunction and CSD (148,155), might play roles in the pathogenesis of migraine in CADASIL.

Although allelic and gene-dosage effects on CSD as well as further modulation by sex hormones in FHM mice translate well into the clinical phenotype of migraine (30,51,108,109), experimental designs and differences between the monogenic FHM1 and common migraine pose challenges on the use of FHM1 mice as a preclinical model. While many FHM patients report similar trigger factors as patients with classical MA (156), patients with FHM appear less sensitive to NTG or CGRP provocation (157) compared to patients with common migraine, raising questions regarding the validity of using FHM1 to model migraine. However, several methodological limitations of NTG or CGRP provocation tests have been raised (18). These include the notion that the sensitivity and specificity of the NTG or CGRP provocation tests are

too low to validate them as accurate diagnostic tools for migraine (18). Also, the finding that many FHM patients report similar trigger factors as MA patients and also develop attacks of common migraine (156) supports the validity of investigating mechanisms of migraine in relation to FHM mutations. Future studies across the different monogenic mouse models could uncover whether migraine-relevant triggers like NTG (as tested for the FASPS mouse model (100)), CGRP or pituitary adenylate cyclase-activating polypeptide (PACAP) (as tested for other transgenic or WT mice (44,45) and rats (158)) yield distinct effects that can give specific insight in the molecular and functional pathways underlying migraine headache.

Usefulness of studying comorbidities of migraine (epilepsy, stroke, depression)

The high comorbidity of migraine with neuropsychiatric disorders such as epilepsy (10,11), stroke (6–9), or depressive disorders (14,15) suggests shared pathogenic mechanisms for these diseases. These shared mechanisms could be genetically encoded or partially exerted by complex genetic-environmental interactions, whereby genetic animal models of migraine provide a chance to investigate underlying pathophysiological changes in vivo.

Epilepsy

Based on genetic and functional studies, migraine and epilepsy display partially overlapping mechanisms related to dysfunction of ion transport, which likely explains why the two diseases are associated (159). Although the neurological symptoms during migraine attacks are quite distinct from those of seizures, hyperexcitability is a key feature in both diseases (11,160,161). From this perspective, comparison of functional effects of pathogenic mutations linked to either migraine, epilepsy, or both diseases can reveal disease mechanisms that may be missed when focusing on only one disease. For FHM1, FHM2 and FHM3, certain mutations are linked to migraine and epilepsy both within families and individual mutation carriers (54,66,68). For FHM1, this is evident for the S218L mutation, with S218L KI mice mimicking the human phenotype of FHM and seizure susceptibility by displaying spontaneous or CSD-induced generalized seizures (101,108), in addition to spontaneous potentially headache-related behavior (126). Cellular studies on excitatory and inhibitory neurons carrying either the mild R192Q or severe S218L mutation suggest that the susceptibility to generalized seizures in S218L KI mice is related to a strongly enhanced excitatory transmission resulting in excessive recruitment of neuronal networks (102,103). It was recently shown in a mouse model for sudden unexpected death in epilepsy (SUDEP) that with a paradigm of induced seizures, fatal outcome was related to brainstem spreading depression (162). The combination of spontaneous fatal seizures (101) and facilitated spread of CSD to subcortical regions in S218L mutants (30) seems to make S218L KI mice well suited for studying mechanisms of seizures and SUDEP in migraine-susceptible brains.

Another example that links mechanisms of migraine with epilepsy comes from the FHM3 L263V mutation, which in patients causes both FHM and epilepsy (68). At the cellular level, the L263V mutation yields a gain-of-function effect, which is believed to largely affect inhibitory neurons (72). For the mutation Q1489K, a gain-of-function was associated with self-limiting hyperexcitability that was hypothesized to promote development of CSD by accumulation of extracellular K^+ (163). Dynamic changes in the activity of the affected neuronal networks and associated ion activity changes (164–167) may determine whether neuronal hyperexcitability results in a seizure or a CSD, as suggested by context-dependent shifts from loss- to gain-of-function effects of FHM3 mutations (168). This could be studied, for example, by combining CSD and seizure induction paradigms in existing FHM1 as well as novel FHM3 mouse models.

Further evidence supporting a pathophysiological connection between migraine and epilepsy is that commonly used antiepileptic drugs such as valproic acid and topiramate are efficacious migraine-preventive drugs (169) and effectively suppress CSD in rats (38,170) and mice (171). Although comparison of antiepileptic drug effects across the monogenic mouse models is currently lacking, this is expected to provide useful insight into the role of neuronal hyperexcitability in increasing CSD susceptibility as well as the comorbid connection between migraine and epilepsy.

Stroke

Epidemiological studies have demonstrated an increased risk of ischemic or hemorrhage stroke in patients with migraine, particularly in otherwise healthy female MA patients younger than 45 years of age (6–9). Neuroimaging studies also showed a high incidence of white-matter hyperintensities or infarct-like lesions in migraineurs, further supporting the concept of an increased cerebral vulnerability to ischemia in migraine-susceptible brains (172,173). A recent large-scale meta-analysis of genome-wide data revealed shared genetic susceptibility between migraine and ischemic stroke, linking the biological basis of these common neurovascular disorders at the population

level (174). However, there is a mechanistic gap in translating these findings to the molecular level. Monogenic migraine models, such as FHM1 and CADASIL transgenic mice, have helped to improve our understanding of the association between migraine and stroke (175). FHM1 R192Q and S218L mutant mice develop larger infarcts than WT mice after experimentally induced transient focal cerebral ischemia (176). The phenotype correlated well with the genotype (S218L worse than R192Q) and showed an allele-dosage effect (homozygotes worse than heterozygotes). A likely explanation is that neuronal hyperexcitability implicated in migraine pathogenesis also increases the vulnerability to ischemic injury. In fact, during experimentally induced cerebral ischemia, faster anoxic depolarization and much more frequent peri-infarct depolarizations (PIDs) develop, leading to exacerbated metabolic supply-demand mismatch and faster infarct growth (176). Treatment with the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 prevented infarct growth and improved outcome to levels observed in WT mice, suggesting CSD as a potential target for preventive or acute stroke treatment (176). Consistent with this notion, chronic treatment with the migraine-prophylactic drugs topiramate or lamotrigine reduced the number of ischemic depolarizations and improved stroke outcomes both in WT and FHM1 KI mice (171). These findings suggest that hyperexcitability resulting from a genetically enhanced glutamatergic neurotransmission might be a common mechanism both for migraine pathogenesis and tissue sensitization to ischemia. The faster and larger neuronal $(Ca^{2+})_i$ surge during CSD in FHM1 mice (as outlined above), together with more severe oligemia and hemoglobin desaturation following CSD (113) may also contribute to the enhanced vulnerability to ischemia in migraine-susceptible brains (176). These findings hold true for the clinical situation in that stroke patients with a history of migraine show an accelerated infarct growth compared to those without a migraine history (177–179). Similarly, and also associated with an increased susceptibility to CSD (148), *Notch3* mutant (R90C) and *Notch3* knockout mice develop larger infarcts with an increased frequency of PIDs upon experimental stroke (139,180).

Depression

A bidirectional association of migraine and depression has been disclosed in longitudinal population-based studies; the relative risk of developing depression in patients with migraine ranged from 2.4 to 5.8, while the risk of developing migraine in patients with depression ranged from 2.8 to 3.5 (14,181,182). In clinic-based studies, patients with chronic migraine showed high occurrence of major depression; in addition, the presence of major depression predicted poor treatment outcome in patients with chronic migraine (183,184). Further underscoring the pathophysiological connection between migraine and depression, antidepressants are widely used for effective migraine prophylaxis (169) and the tricyclic antidepressant amitriptyline has been shown to suppress CSD susceptibility in rats (38). Genetically engineered migraine mouse models have not been examined for their susceptibility to major depression. Since there are well-established paradigms to study major depressive disorder-related neurobehavioral traits in non-human species (185), it would be feasible to adopt some of these paradigms in genetic animal models of migraine to examine the comorbidity between migraine and depression. For example, one might investigate whether transgenic migraine models display behavioral or functional changes indicative of depression using chronic psychosocial stress models or modulation of circuits known to induce depressive behavior (186). To explore the bidirectional association between migraine and depression, one could also study whether genetically engineered mice with depressive features (186) exhibit increased CSD susceptibility and behavior related to pain such as craniofacial grimacing upon NTG or CGRP infusion, and explore responses to antidepressants known to be effective in migraine prophylaxis.

Future directions

Promising experimental strategies for unraveling migraine mechanisms using genetic animal models

As outlined above, animal models for monogenic forms of migraine are valuable tools to unravel mechanisms for clinically important questions, such as how migraine aura links to migraine headache, which factors lead to the development of migraine pain, and how the presumed gene-environment interplay relates to clinical phenotypes. Much of the available research in mouse models has focused on CSD as the mechanism underlying aura and perhaps headache. However, while migraine aura is a predominant symptom in FHM1, aura occurs in only about one-third of migraine patients, and it remains a matter of debate whether patients with migraine without perceived aura exhibit CSD as well that remains clinically silent. Therefore, more research is needed to explore other activators of the TVS than CSD, and gain insight in trigeminovascular processing in animal models of migraine such as CGRP models (44,45) and novel models of migraine in which aura appears as a less-prominent feature (148,152). Future studies should combine experimental strategies to test different modalities in multiple mouse

mutants, in order to evaluate numerous migraine symptoms including combinations of models for aura, photo/phonophobia, and headache (e.g. CSD, NTG, epidural inflammatory soup infusion).

Much is expected from the dissection of the biomolecular basis of the genetic-environmental interplay by employment of cutting edge systems biology technologies like (epi)genomics, metabolomics or proteomics. Studies on monogenic migraine animal models seem especially helpful for characterizing complex interactions between genetic predisposition and epigenetic modifications at the organism level. A recent review has comprehensively summarized how epigenetic factors might influence migraine pathophysiology (187). Disease-modulating factors such as sex hormones, stress, childhood abuse, or preventive drugs have been shown to be important modifiers for epigenetic processes such as DNA methylation and chromatin remodeling (188–192). For example, the migraine-prophylactic drug and CSD inhibitor valproic acid is a known inhibitor of histone deacetylases (HDACs) (192). Modulating factors known to contribute to migraine onset, presentation, and evolution (34), both external (e.g. sensory triggers, environmental changes, hormonal treatment) and internal (e.g. hormone fluctuations, metabolic changes), can have significant effects at the epigenetic level. Using innovative technologies like molecular imaging (193), such effects could be identified at the cellular or tissue-specific level in genetic animal models of migraine. Nonetheless, biological roles of proposed epigenetic regulation in migraine pathophysiology have yet to be confirmed. It remains a question whether epigenetic changes contribute to the dynamic changes underlying the cyclic occurrence as well as chronification of migraine.

To investigate consequences of CSD at the biomolecular level, large-scale matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) has been employed in the FHM1 transgenic mouse model (194). With co-registration to the Allen Brain Atlas reference atlas of the mouse brain (195), molecular signatures revealed by MSI have been compared across distinct cortical and subcortical brain regions. The study disclosed significant mass spectral feature changes in the cortex (146 and 377 Da) and thalamus (1820 and 1834 Da) of the CSD-affected hemisphere of FHM1 transgenic mice but not WT, indicating that CSD-induced metabolite changes are genotype specific (194). Capillary electrophoresis-mass spectrometry recently demonstrated plasma metabolic changes following CSD in FHM1 mice. The study identified a significant reduction in the level of plasma lysine and a significant increase in the plasma level of its by-product pipecolic acid after CSD in FHM1 compared to WT mice, suggesting a compensatory increase in GABAergic neurotransmission in the presence of enhanced excitatory neurotransmission (196). Exploring biomolecular changes in FHM1 and other mouse models may improve our understanding about pathways linking CSD to migraine, and studying biomolecular effects of TVS activation using, for example, NTG or inflammatory soup could provide valuable insight in molecular pathways leading to migraine headache.

The implementation of optogenetic technology in brain research (197) is ideally suited to further our mechanistic insight in the contributions of the brain's different cellular and network components to migraine pathophysiology. In freely behaving mice, optogenetics has already been applied for selective optical control of nociception and central sensitization (198). Transgenic mice in which light-sensitive channelrhodopsin-2 (ChR2) channels were expressed in $Na_V1.8$-positive sensory neurons displayed pronounced nociceptive behavior in response to blue light illumination on the skin, which causes ChR2-containing neurons to depolarize and to remotely stimulate nociceptive fibers. The observation that optogenetics can be used to noninvasively trigger CSD events in transgenic mice expressing ChR2 channels in the cortex (19) provides a technical basis to study the dynamic changes in neuronal and non-neuronal activities believed to underlie initiation of CSD and subsequent migraine headache mechanisms. If network changes predictive of CSD can be identified, these could serve as functional biomarkers for upcoming CSD events (199), and could be therapeutically targeted. Ultimately, such predictive biomarkers might be used for brain activity-guided optogenetic feedback (200), whereby biomarker data would provide the input to optogenetically suppress CSD-initiating mechanisms, in a closed-loop manner. Ultimately, it will be extremely useful to integrate data on brain activity and behavior with high-throughput biomolecular information from (epi)genomics, metabolomics and proteomics studies. The recent demonstration that gene expression and epigenetic changes could be modulated in real time by optogenetic control using light-inducible transcriptional effectors (201) opens the possibility to directly investigate biological effects of (epi)genetic changes both at functional and biomolecular levels.

Opportunities for common migraine

Clinical evidence suggests that mechanisms identified from studies in genetic animal models for monogenic migraine subtypes, such as impaired ion homeostasis, enhanced glutamatergic neurotransmission and the related increase in CSD susceptibility, might also be involved in common forms of migraine. Risk alleles

identified from GWAS support a role of cerebral hyperexcitability in common migraine (202,203), but these mechanisms are difficult to evaluate in vivo. To explore the molecular basis of such risk alleles for common migraine by genetically engineered mouse models is not straightforward given the small effect size and potentially large number of risk genes, as well as their complex interaction with environmental factors. A reasonable start might be to focus on those genetic variants that have been successfully replicated in multiple GWAS (e.g. *TRPM8* or *LRP1*) (202), or on variants already known to affect similar pathways or exert similar function (e.g. oxidative stress) (202). It might be predicted that future work could aim at interrogating multiple genes with different functions at the same time in vivo, if state-of-the-art genetic editing techniques are ready to efficiently generate proper animal lines.

Key findings

Transgenic mice carrying human monogenic migraine mutations:
- recapitulate clinical phenotypes of migraine,
- have helped to elucidate mechanisms of migraine and its association with stroke,
- display an enhanced susceptibility to spreading depression, the neurobiological event underlying migraine that increases vulnerability to cerebral ischemia.

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