Migraine Mutations Increase Stroke Vulnerability by Facilitating Ischemic Depolarizations

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Background—Migraine is an independent risk factor for stroke. Mechanisms underlying this association are unclear. Familial hemiplegic migraine (FHM), a migraine subtype that also carries an increased stroke risk, is a useful model for common migraine phenotypes because of shared aura and headache features, trigger factors, and underlying glutamatergic mechanisms.

Methods and Results—Here, we show that FHM type 1 (FHM1) mutations in CaV2.1 voltage-gated Ca2+ channels render the brain more vulnerable to ischemic stroke. Compared with wild-type mice, 2 FHM1 mutant mouse strains developed earlier onset of anoxic depolarization and more frequent peri-infarct depolarizations associated with rapid expansion of infract core on diffusion-weighted magnetic resonance imaging and larger perfusion deficits on laser speckle flowmetry. Cerebral blood flow required for tissue survival was higher in the mutants, leading to infarction with milder ischemia. As a result, mutants developed larger infarcts and worse neurological outcomes after stroke, which were selectively attenuated by a glutamate receptor antagonist.

Conclusions—We propose that enhanced susceptibility to ischemic depolarizations akin to spreading depression predisposes migraineurs to infarction during mild ischemic events, thereby increasing the stroke risk. (Circulation. 2012;125:335-345.)

Key Words: calcium channels ■ cortical spreading depression ■ migraine disorders ■ stroke

Migraine is the most common neurological condition affecting young to middle-age adults. Up to one third of migraineurs experience transient neurological symptoms called aura. Migraine, particularly with aura, is associated with increased stroke risk both during and between attacks, especially in women.1–4 The biological basis for this association is unclear. Familial hemiplegic migraine (FHM), a migraine subtype that also carries an increased stroke risk, is a useful model for common migraine phenotypes because of shared aura and headache features, trigger factors, and underlying glutamatergic mechanisms.

FHM type 1 (FHM1) mutations in CaV2.1 voltage-gated Ca2+ channels render the brain more vulnerable to ischemic stroke. Compared with wild-type mice, 2 FHM1 mutant mouse strains developed earlier onset of anoxic depolarization and more frequent peri-infarct depolarizations associated with rapid expansion of infarct core on diffusion-weighted magnetic resonance imaging and larger perfusion deficits on laser speckle flowmetry. Cerebral blood flow required for tissue survival was higher in the mutants, leading to infarction with milder ischemia. As a result, mutants developed larger infarcts and worse neurological outcomes after stroke, which were selectively attenuated by a glutamate receptor antagonist.

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FHM type 1 (FHM1) is caused by mutations in the CACNA1A gene, which encodes the pore-forming α1A subunit of neuronal Ca2.1 voltage-gated Ca2+ channels.9 Presynaptic Ca2.1 channels are major regulators of excitatory neurotransmitter release. FHM1 mutant channels open with smaller depolarizations and stay open longer,10 which augments presynaptic Ca2+ entry and glutamate release, thereby enhancing brain excitability.11 Transgenic mice expressing the human R192Q or S218L FHM1 mutation show an increased susceptibility to spreading depolarization, the electrophysiological substrate for migraine, and display
characteristic clinical features of FHM such as transient hemiplegia. Glutamatergic mechanisms and hyperexcitability also have been implicated in the pathogenesis of common forms of migraine. Genetic support for this link was recently obtained in a genome-wide association study identifying the astrocyte elevated gene 1 (AEG1), encoding a regulator of glial glutamate transporter EAAT2, as the first migraine gene.

Glutamate excitotoxicity also plays a pivotal role in the pathogenesis of stroke. Therefore, we hypothesized that genetic mutations conferring cerebral hyperexcitability and migraine susceptibility increase the vulnerability to ischemic stroke, as 1 mechanism to explain the migraine-stroke association. We tested this hypothesis using CaV2.1 S218L and R192Q transgenic mouse models of FHM1. The results reveal electrophysiological and hemodynamic mechanisms that accelerate hyperacute stroke evolution and worsen ischemic outcome in FHM1 mutants and suggest a pivotal role for enhanced glutamatergic transmission in increasing the vulnerability to ischemic stroke in susceptible migraineurs.

Methods

Experimental Animals

Experimental procedures were approved by the institutional review boards. A total of 267 male and female mice were used. Transgenic knock-in Caenorhabditis elegans (HOM) or heterozygous (HET) for R192Q or S218L FHM1 mutations were generated by a gene targeting approach. The R192Q mutant strain was compared with their wild-type (WT) littermates. Because stroke risk is highest in young adult migraineurs, mice were studied between 2 and 6 months of age. All experiments were carried out by blinded investigators, and confirmatory genotyping was done.

Systemic Physiological Monitoring

Arterial pH, PO2, PCO2, and blood pressure were measured via a femoral artery catheter under isoflurane anesthesia (2.5% induction, 1.5% maintenance, in 70% N2O and 30% O2; Table I in the online-only Data Supplement). Rectal temperature was controlled at 37°C during ischemia, and intermittent monitoring was continued for 6 hours in a subset of mice.

Transient Filament Occlusion of the Middle Cerebral Artery

A nylon monofilament was inserted into the internal carotid artery via the external carotid artery followed by reperfusion after 30 or 60 minutes under isoflurane anesthesia (2.5% induction, 1.5% maintenance, in 70% N2O and 30% O2; Table I in the online-only Data Supplement). Rectal temperature was controlled at 37°C during ischemia, and intermittent monitoring was continued for 6 hours in a subset of mice.

Magnetic Resonance Imaging

Apparent diffusion coefficient maps were acquired under isoflurane anesthesia with a 9.4-T magnetic resonance imaging (MRI) scanner (Bruker Biospin, Inc, Billerica, MA) 30 and 60 minutes after filament occlusion (repetition time/echo time, 3000/27 milliseconds; b, 154 and 1294 s/mm2; in-plane resolution, 180 × 180 μm2; slice thickness, 1 mm; number of averaging, 8). Means and SDs of the apparent diffusion coefficient in the cortex, striatum, hippocampus, and thalamus were extracted from the normal hemisphere, and thresholds were defined as mean minus 2 SDs to calculate lesion volumes. Normal systemic physiological parameters were confirmed under simulated MRI conditions in a separate group of mice (not shown).

Electrophysiological Recordings

After filament occlusion, isoflurane-anesthetized mice were intubated and ventilated, and the femoral artery was catheterized for blood pressure and blood gas monitoring. Two intracortical glass micropipettes were placed, and extracellular recordings (depth, 250 μm) were started within 15 minutes after the onset of ischemia and continued for ~2 hours.

Receptor Autoradiography

The density and distribution of glutamate and GABA A receptors and glutamate reuptake sites were assessed on 10-μm frozen sections with tritium-sensitive storage phosphor screens (GE Healthcare) as described.

Laser Speckle Flowmetry

Spontaneously breathing mice (S218L HOM) were anesthetized with isoflurane as above, and the femoral artery was catheterized for blood pressure and gas measurements. Mice were placed in a stereotaxic frame; a temporal burr hole (2-mm diameter) was drilled above the zygomatic arch; and the distal middle cerebral artery was occluded with a microvascular clip for 60 minutes. Cortical perfusion was imaged during distal middle cerebral artery occlusion with laser speckle flowmetry through intact skull. Cerebral blood flow (CBF) changes were calculated for each pixel relative to the preischemic baseline, and the area of cortex with residual CBF ≤30% was determined by thresholding. Neurological outcomes and infarcts were assessed 48 hours later as described above. In addition, the CBF threshold for tissue viability was estimated by superimposing the images of CBF and infarct. In the R192Q HOM strain, mice were intubated and ventilated to ensure normal arterial blood gas values, precluding survival for neurological and infarct assessment in this strain.

Anatomic Analysis of the Circle of Willis and Pial Collaterals

Mice were transcardially perfused with carbon black. The diameter of cerebral arteries, patency of the posterior communicating artery, and number of pial arterial anastomoses between the anterior, posterior, and middle cerebral arteries and their distance from midline were determined.

Absolute Resting CBF

Mice were anesthetized with α-chloralose (50 mg/kg) and ventilated. The femoral artery and external jugular vein were cannulated. Arterial blood was withdrawn continuously (0.3 mL/min). N-isopropyl- [methyl-1,3-14C]-p-iodoamphetamine (1 μCi) was injected in 0.1 mL saline over 10 seconds. Twenty seconds after injection, the animal was decapitated, and the blood withdrawal was terminated simultaneously. The brain was removed, frozen, and dissected. CBF was calculated from the radioactivity in tissue and blood measured by liquid scintillation spectrometry.

Statistical Analysis

Data were analyzed with SPSS (version 11.0) and are presented as mean ± SD or median and interquartile range. We used the χ2 test to compare proportions, ANOVA to compare mean values of...
Results

Enhanced Susceptibility to Anoxic and Peri-Infarct Depolarizations

Anoxic depolarization is characterized by a sudden loss of membrane ionic gradients, uncontrolled glutamate release, and cell swelling, triggered by the failure of Na\(^+/\)K\(^+\) ATPase under ischemic conditions. We found significantly earlier onset of anoxic depolarization in FHM1 mutants after fMCAO by monitoring its vasoconstrictive effect on cerebral vasculature.\(^{18,19}\) Importantly, the magnitude of CBF reduction in the ischemic core did not differ among groups in this fMCAO model, eliminating the possibility that faster anoxic depolarization rates were due to more severe ischemia (residual CBF, 10% to 17% of baseline in both S218L and R192Q; \(P=0.634\) and 0.599, respectively; data not shown).

PIDs are recurrent propagating depolarization waves akin to spreading depression that exacerbate the metabolic mismatch in penumbra and promote infarct growth during hyperacute stroke.\(^{18-21}\) We reasoned that FHM1 mutations that enhance spreading depression susceptibility\(^{11-14}\) might also facilitate the occurrence of PIDs. Using intracortical microelectrode recordings during fMCAO, we indeed found a 2-fold increase in the frequency of PIDs in mutants over WT \((P<0.001; n=5\) each). Together, these data suggest that genetically enhanced susceptibility to spreading depression\(^{11-14}\) facilitates the occurrence of anoxic depolarization and PIDs during acute stroke as a novel mechanism to explain increased stroke vulnerability in migraineurs.

Rapid Growth of Hyperacute Ischemic Core on MRI

To assess whether enhanced susceptibility to anoxic depolarization and PIDs accelerates the hyperacute stroke evolution in FHM1 mutants, we performed serial diffusion-weighted MRI during fMCAO. Reduced apparent diffusion coefficient values on diffusion-weighted MRI reflect anoxic depolarization, loss of transmembrane ionic gradients, and cell swelling (ie, ischemic core). We found that the apparent diffusion coefficient lesion volumes expanded more rapidly in FHM1 mutant strains compared with WT controls (Figure 2).
though larger apparent diffusion coefficient lesion volumes were due primarily to more severe cortical involvement, the hyperacute lesion also encompassed the hippocampus and thalamus in S218L mutants.

**Larger Perfusion Deficit During Hyperacute Stroke**

Ischemic depolarizations compromise residual CBF within the territory supplied by the occluded artery via vasoconstrictive (ie, inverse) neurovascular coupling\(^{18,19}\) as a major determinant of outcome in cerebral ischemia. Using laser speckle flowmetry, we found larger cortical perfusion deficits after distal middle cerebral artery occlusion in FHM1 mutants (Figure 3A and 3B; only S218L shown), associated with an increased frequency of PIDs (0.9±0.3 versus 4.6±1.2 PIDs per hour in WT and S218L HOM, respectively; Figure 3C) that circled around the hypoperfused core in 38% of S218L mutants but not in the WT (movies I and II in the online-only Data Supplement).\(^{22}\) In fact, higher PID frequencies were associated with larger cortical CBF deficits (Figure 3D), bigger infarcts (Figure 3E and 3F), and worse neurological outcomes (deficit score, 1 [interquartile range, 1–1] versus 0 [interquartile range, 0–0.25] in S218L HOM and WT mice, respectively; \(P=0.019\)). These data suggest that ischemic depolarizations adversely influence the perfusion deficits and exacerbate the metabolic and \(O_2\) supply-demand mismatch in FHM1 mutants, in part via vasoconstrictive (ie, inverse) neurovascular coupling as an additional hemodynamic mechanism for infarct growth.\(^{18,19,23}\)

Importantly, we found no difference in absolute resting CBF values between WT and R192Q HOM mice in the cortex, striatum, and cerebellum using the \(^{13}C\)iodoamphetamine method (Table II in the online-only Data Supplement), indicating that differences in pres ischemic resting CBF did not influence our measurements. We also confirmed this in WT and S218L HOM mice under isoflurane anesthesia using the correlation time values obtained by laser speckle imaging, which allow direct comparison of resting CBF among groups of mice (data not shown).\(^{24,25}\) Moreover, the incidence of incomplete circle of Willis, the diameter of its major branches, and the number and location of pial arterial anastomoses did not differ between WT and S218L HOM mice, suggesting that developmental differences in cerebrovascular anatomy did not contribute to worse perfusion deficits in the mutant mice (Figure I and Table III in the online-only Data Supplement).
Higher CBF Threshold for Tissue Survival

To determine the critical tissue perfusion level below which infarction ensued (ie, viability threshold), we calculated the regional CBF at the infarct margin by spatially coregistering the laser speckle perfusion map during distal middle cerebral artery occlusion (dMCAO) with the infarct that developed 48 hours later (Figure 4). We found that cortical tissue in S218L HOM mutants required a higher CBF level for survival compared with WT mice (42±3% versus 35±2% of baseline CBF, respectively; \( P = 0.048 \)). These data underscore the importance of parenchymal mechanisms such as neuronal hyperexcitability and ischemic depolarizations as the main cause for increased vulnerability to ischemic stroke in FHM1 mutants independently of the severity of CBF deficit.

Worse Stroke Outcomes

Enhanced susceptibility to anoxic depolarization and PID and accelerated hyperacute infarct growth with more severe CBF deficits translated into worse stroke outcomes in FHM1 mutants. Transient fMCAO for 1 hour produced larger
infarcts in both S218L and R192Q mutant mice compared with their WT controls (Figure 5A). Larger infarcts reflected predominantly more severe cortical involvement in both mutants (>70% of total infarct volume); however, the incidence of hippocampal or thalamic infarction also tended to be higher in the S218L mutant (present in 33% of S218L HET mice compared with 13% of WT; \( P < 0.1 \); data not shown), consistent with a higher incidence of subcortical infarction observed on MRI in this strain (see above). Functional outcomes, assessed with a combined death and neurological disability score as a clinically relevant end point, were worse in mutants compared with WT (the Table). Indeed, the mortality rate was significantly higher in the S218L mutants, reaching 100% in the HOM within 24 hours after stroke onset (Figure IIa in the online-only Data Supplement). The timing of death after stroke was variable (12 ± 3 hours after stroke onset) and was not associated with overt seizure activity. Immediate postmortem examination revealed 2-fold larger infarcts in S218L HOM compared with WT mice euthanized at the same time point of death of each mutant after 60 minutes of fMCAO (95 ± 15 versus 44 ± 7 mm³, respectively; \( n=5 \) and 4; \( P=0.031 \)), suggesting that selection bias resulting

Figure 4. Elevated blood flow threshold for tissue survival in FHM1 mutant mice. A, Representative laser speckle contrast images (LSCI) during distal middle cerebral artery occlusion (dMCAO; left) and 2,3,5-triphenyltetrazolium chloride (TTC)–stained brain showing the infarct 48 hours after 60 minutes of dMCAO (right) are shown for wild-type (WT) and S218L homozygous (HOM) mice. Imaging field was positioned as shown in Figure 3A. Images were spatially coregistered through the use of surface landmarks. Line profiles (blue and green oblique lines, labeled in mm) were drawn between lambda and the clip occluding the middle cerebral artery branch (yellow arrowheads). B, For each animal, cortical blood flow (CBF) was plotted along these line profiles as a function of distance from lambda using laser speckle images, and the blood flow level corresponding to the infarct edge was determined (red dotted lines). This value represented the CBF threshold for viability, below which the tissue infarcted in each mouse. C, The average viability threshold was significantly higher in S218L HOM mutants vs WT controls (\( P=0.048 \)), indicating that FHM1 mutant brains are more vulnerable to ischemia and require higher blood flow to survive. The numbers of mice are shown on each bar. *\( P<0.05 \) vs WT.
from high mortality in the mutants diminished the strain differences in outcome. These data were excluded from the overall comparisons among genotypes (Figure 5) because of variable time of death. Ischemic brain swelling tended to be more severe in the mutants in proportion to the actual infarct volume and might have contributed to the high mortality in the S218L mutants.

To mimic transient ischemic attacks and to circumvent the high mortality rate in S218L HOM mice, we subjected this mutant strain to 30 minutes of fMCAO. With a shorter duration of ischemia, we did not detect overt infarcts in 27% of WT mice using TTC staining, whereas all S218L HET and HOM mutant mice developed conspicuous territorial infarcts (P<0.077). Selective ischemic changes in scattered neurons were nevertheless present on histological examination of brains without an overt infarct (not shown). Infarct volumes were once again larger in the S218L mutants compared with WT mice (Figure 5B). The volume of subcortical infarction, limited to the striatum in this shorter ischemia model, was also larger in the S218L HET compared with WT mice (17±4 and 5±1 mm³, respectively, in males, P=0.002; 16±3 and 4±2 mm³, respectively, in females, P=0.013). Despite the shorter ischemia duration, mortality was still high in the S218L HOM mutants (75%), but all HET mutants survived for at least 24 hours and showed a trend for worse functional outcome compared with WT (P=0.086; the Table).

Because classic migraine, sporadic migraine, and FHM are more prevalent in women of reproductive age 5,26–29 and because susceptibility to spreading depression is higher in female FHM1 mutant mice compared with males,13,30 we also studied female mice and found an even more striking increase in infarct volumes in S218L HET compared with WT mice (Figure 5B). The volume of subcortical infarction, limited to the striatum in this shorter ischemia model, was also larger in the S218L HET compared with WT mice (17±4 and 5±1 mm³, respectively, in males, P=0.002; 16±3 and 4±2 mm³, respectively, in females, P=0.013). Despite the shorter ischemia duration, mortality was still high in the S218L HOM mutants (75%), but all HET mutants survived for at least 24 hours and showed a trend for worse functional outcome compared with WT (P=0.086; the Table).

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5A), and functional outcome was improved only in S218L HET compared with only 23% in WT (Figure 45B). To assess long-term tissue and neurological outcome (2 weeks) in FHM1 mutants, we subjected female S218L HET and WT mice to 30 minutes of fMCAO. However, we observed >60% mortality in the mutants predominantly between 24 and 96 hours, which precluded outcome comparisons between the mutant and WT strains at this late time point (Figure IIb in the online-only Data Supplement). Together, these data indicate that mice expressing FHM1 mutations are particularly susceptible to infarction when challenged by cerebral arterial occlusion.

Enhanced Neuroprotective Efficacy of Glutamate Receptor Antagonist MK-801

FHM1 mutations enhance glutamate release, and glutamate plays a pivotal role in spreading depression and ischemic depolarizations, as well as in excitotoxic cell death, mainly via the N-methyl-D-aspartate (NMDA) subtype of receptors. Therefore, we tested whether enhanced glutamatergic activity in FHM1 mutants is responsible for their vulnerability to infarction. Preischemic treatment with the NMDA receptor inhibitor MK-801 abolished the differences in stroke phenotype between genotypes. MK-801 reduced infarct volume by 45% in S218L HET compared with only 23% in WT (Figure 5A), and functional outcome was improved only in S218L mutants (the Table).

As an important control, we also examined the density of glutamate and GABA_A binding sites in the FHM1 mutant and WT mice using quantitative in vitro autoradiography and did not find overt differences (Figure III and Table IV in the online-only Data Supplement). These data are consistent with recent proteomics analysis of cortical synapses in this mutant and suggest that enhanced susceptibility to ischemic depolarizations in FHM1 mutants is unlikely to reflect changes in neurotransmitter receptors and reuptake mechanisms.

Discussion

Our data provide a novel mechanism to explain the higher incidence of ischemic stroke in migraineurs. Two genetic mouse models expressing FHM1 mutations were at risk of developing large infarcts and worse neurological outcomes after transient focal cerebral ischemia. Consistent with the higher stroke risk in women compared with men with migraine with aura, we found more striking increases in infarct volume in female mutants compared with males. Faster anoxic depolarization rates, more frequent PIDs, and enhanced neuroprotective efficacy of the NMDA antagonist MK-801 in the mutants implicated glutamatergic neuronal hyperexcitability as 1 mechanism, and larger perfusion defects linked to ischemic depolarizations implicated vasoconstrictive neurovascular coupling as another. Therefore, neuronal and vascular mechanisms together render migraineurs more vulnerable to cerebral infarction on ischemia.

The data have clinical implications. In susceptible migraineurs, increased sensitivity to ischemia may predispose to strokes during mild ischemic events, which remain clinically silent or manifest only as transient ischemic attacks in nonmigraineurs. Moreover, elevated CBF threshold for viability, a sign of increased vulnerability to ischemia, may promote rapid infarct expansion into tissue with milder perfusion deficits, diminish salvageable tissue at risk (ie, ischemic penumbra), and shorten the therapeutic window of acute stroke interventions in migraineurs. Lastly, higher mortality among the S218L mutants may have implications for malignant infarcts, large supratentorial strokes characterized by progressive loss of consciousness over 48 hours with up to 80% mortality if untreated. To date, there has been no reliable predictor for malignant infarction, and history of migraine with aura (or its genetic determinants) may be 1 such marker increasing the risk of stroke progression and, in case of large territorial infarcts, the risk of death, as has recently been reported for hemorrhagic stroke in migraineurs.

Mechanisms of Ischemic Vulnerability in FHM1 Mice

Biological mechanisms underlying the association between migraine and stroke are unknown, although in both diseases dynamic interactions among the constituents of the neurovascular unit are important to the pathophysiology. At the onset of cerebral ischemia, the gradual failure of Na^+/K^+-ATPase causes a slow rise in extracellular K^+ and loss of neuronal membrane potential until a critical threshold for initiation of
anoxic depolarization is reached.\textsuperscript{35,36} FHM1 mutations shift the Ca\(_{\text{v}}\)2.1 channel opening voltage to more negative membrane potentials so that channels open with smaller depolarizations, triggering glutamate release, which can explain faster anoxic depolarization onset in the mutants. Glutamate is also critical for PIDs, which are spreading depolarization waves triggered in ischemic penumbra. Therefore, enhanced release in ischemic penumbra can also explain higher PID frequencies in FHM1 mutants.\textsuperscript{41} Delayed Ca\(_{\text{v}}\)2.1 channel inactivation may exacerbate the excitotoxicity by prolonging the Ca\(^{2+}\) influx and glutamate release during PIDs in penumbra. Moreover, PIDs exacerbate the metabolic mismatch in penumbra by stimulating O\(_{2}\) and glucose consumption and by worsening tissue perfusion via vasoconstrictive (ie, inverse) neurovascular coupling,\textsuperscript{18,19,23} particularly when they occur with high frequency and in clusters, as observed in FHM1 mutants.\textsuperscript{19,37–39} With each PID, more of the penumbra is incorporated into the core, accounting for concentric infarct growth over time.\textsuperscript{22,40–42} PIDs occur frequently in human brain after ischemic or hemorrhagic stroke and head trauma and appear to worsen patient outcomes, similar to experimental stroke.\textsuperscript{38,43–45} Hence, migraine with aura may be a risk factor for increased occurrence of PIDs and worse outcomes in human stroke, as recently suggested in subarachnoid hemorrhage.\textsuperscript{46}

### Association Between Migraine and Stroke

Our data in FHM1 mutant mice support shared genetic risk factors enhancing susceptibility to spreading depression as a mechanism to explain the migraine-stroke association. Shared genetic factors enhancing susceptibility to migraine and stroke such as NOTCH3 mutations in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy have been described.\textsuperscript{47–50} Indeed, NOTCH3 mutations, despite being exclusively expressed in vascular smooth muscle cells, augment susceptibility to spreading depression.\textsuperscript{51} Mutations associated with FH2 and FH3 are also predicted to enhance neuronal excitability and spreading depression susceptibility possibly via glutamatergic mechanisms.\textsuperscript{52} Glutamatergic mechanisms and hyperexcitability also are implicated in common forms of migraine by recent studies linking AEG1, encoding a regulator of glial glutamate transporter EAAT2, and KCNK18, encoding the TRESK potassium channel, to migraine.\textsuperscript{15,16} Vascular mechanisms (eg, endothelial dysfunction) have also been implicated in increased stroke risk in migraineurs.\textsuperscript{53,54} Indeed, functional Ca\(_{\text{v}}\)2.1 channel expression has been reported in renovascular smooth muscle cells,\textsuperscript{55,56} but whether FHM1 mutations alter cerebrovascular physiology is not known. Together with parenchymal mechanisms that enhance vulnerability to perfusion deficits, vascular mechanisms might further augment stroke risk in migraineurs. For example, highly focal and mild ischemic vascular events such as microembolism\textsuperscript{57} may trigger spreading depression more readily in migraineurs highly susceptible to ischemic depolarizations, providing a possible explanation for the origin of a subset of migraine auras.

### Conclusions

A monogenic determinant of migraine with aura increases stroke vulnerability via glutamatergic mechanisms that enhance susceptibility to ischemic depolarizations akin to spreading depression and accelerate stroke evolution. Hence, our data put FHM1 mutations among the shared genetic determinants of migraine with aura and stroke. More work is needed to extrapolate these data to other monogenic syndromes and to the more common and genetically more complex forms of migraine with aura and to determine whether targeting hyperexcitability and spreading depression such as migraine prophylaxis\textsuperscript{58} confers ischemic protection in susceptible mouse strains or in migraineurs.

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### Disclosures

None.

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CLINICAL PERSPECTIVE

Our study establishes a mechanism that links migraine and stroke, 2 highly prevalent and debilitating diseases. Migraine is a well-recognized stroke risk factor. Although its prevalence is on par with other stroke risk factors such as diabetes mellitus and hypertension, there has been little insight into the mechanism of the migraine-stroke association. Here, we present compelling evidence indicating that glutamatergic hyperexcitability associated with migraine mutations renders the brain more susceptible to ischemic depolarizations. As a result, the minimum critical level of blood flow required for tissue survival (ie, viability threshold) is elevated and infarction ensues, even in mildly ischemic tissues. This represents a paradigm shift in the search for a mechanism for increased stroke risk in migraineurs and differs radically from those previously postulated on the basis of clinical data alone. Our conclusions are based on optical and magnetic resonance imaging and electrophysiological recordings in transgenic mouse models for familial hemiplegic migraine type 1, a monogenic migraine syndrome (mutations in Ca(2.1) channels) that has been a model for common but genetically complex forms of migraine based on shared clinical features, glutamatergic mechanisms, and elevated stroke risk. Clinical implications include a shorter therapeutic window for acute stroke interventions in migraineurs because of accelerated loss of potentially salvageable penumbra. Furthermore, migraine prophylaxis may reduce stroke risk by suppressing cerebral hyperexcitability, and antithrombotic prophylaxis may be indicated in susceptible migraineurs because they are more likely to have infarcts if and when they develop cerebral ischemic events.
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### Supplemental Table 1. Age, body weight and systemic physiological parameters.

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<th>Age (mo)</th>
<th>Weight (g)</th>
<th>BP (mmHg)</th>
<th>Arterial pH</th>
<th>Arterial pCO$_2$ (mmHg)</th>
<th>Arterial pO$_2$ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R192Q</td>
<td>WT</td>
<td>11</td>
<td>3.8±0.2</td>
<td>27±1</td>
<td>84±2</td>
<td>7.36±0.01</td>
<td>33±1</td>
</tr>
<tr>
<td></td>
<td>HOM</td>
<td>16</td>
<td>4.5±0.4</td>
<td>27±1</td>
<td>90±2</td>
<td>7.36±0.01</td>
<td>36±4</td>
</tr>
<tr>
<td>S218L</td>
<td>WT</td>
<td>23</td>
<td>4.4±0.2</td>
<td>27±1</td>
<td>89±3</td>
<td>7.36±0.01</td>
<td>37±1</td>
</tr>
<tr>
<td></td>
<td>HOM</td>
<td>20</td>
<td>3.9±0.2</td>
<td>26±1</td>
<td>97±3</td>
<td>7.38±0.01</td>
<td>35±1</td>
</tr>
</tbody>
</table>

There was no statistically significant difference between WT and HOM mutant of each strain. Systemic physiology was measured via a femoral artery catheter in non-survival experiments only, to minimize morbidity in survival groups.
Supplemental Table 2. Resting cerebral blood flow.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>R192Q HOM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>130 ± 28</td>
<td>130 ± 17</td>
<td>0.993</td>
</tr>
<tr>
<td>Striatum</td>
<td>139 ± 29</td>
<td>146 ± 16</td>
<td>0.649</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>150 ± 24</td>
<td>162 ± 15</td>
<td>0.344</td>
</tr>
</tbody>
</table>

Values are absolute blood flow in ml/100g/min determined by [14C]-iodoamphetamine radioactive tracer method. N=5 each genotype.
### Supplemental Table 3. Cerebrovascular anatomy.

<table>
<thead>
<tr>
<th></th>
<th>WT (n=7)</th>
<th>S218L HOM (n=7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICA diameter (μm)</td>
<td>132 ± 5</td>
<td>128 ± 2</td>
<td>0.487</td>
</tr>
<tr>
<td>MCA diameter (μm)</td>
<td>111 ± 5</td>
<td>103 ± 3</td>
<td>0.230</td>
</tr>
<tr>
<td>ACA diameter (μm)</td>
<td>113 ± 5</td>
<td>105 ± 6</td>
<td>0.338</td>
</tr>
<tr>
<td>PCA diameter (μm)</td>
<td>115 ± 4</td>
<td>111 ± 2</td>
<td>0.391</td>
</tr>
<tr>
<td>BA diameter (μm)</td>
<td>163 ± 5</td>
<td>160 ± 7</td>
<td>0.707</td>
</tr>
<tr>
<td>PComA diameter (μm)</td>
<td>31 ± 3</td>
<td>29 ± 7</td>
<td>0.806</td>
</tr>
<tr>
<td>Number of mice with unilaterally absent PComA (n)</td>
<td>1</td>
<td>3</td>
<td>0.237</td>
</tr>
<tr>
<td>Number of pial anastomoses between MCA and ACA (n)</td>
<td>6.9 ± 0.9</td>
<td>6.6 ± 1.0</td>
<td>0.692</td>
</tr>
<tr>
<td>Anastomoses distance from midline (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rostral 1/3 of cortex</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>0.235</td>
</tr>
<tr>
<td>Middle 1/3 of cortex</td>
<td>1.8 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>0.383</td>
</tr>
<tr>
<td>Caudal 1/3 of cortex</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>0.149</td>
</tr>
</tbody>
</table>

Data were obtained using intracardiac carbon black infusion. Please also see Supplementary Figure 1 for representative images, abbreviations and the method of measurements.
Supplemental Table 4. Quantitative receptor autoradiography.

<table>
<thead>
<tr>
<th></th>
<th>EAAT</th>
<th>AMPA</th>
<th>KA</th>
<th>NMDA</th>
<th>GABA&lt;sub&gt;A&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WT</td>
<td>R192Q HOM</td>
<td>WT</td>
<td>R192Q HOM</td>
<td>WT</td>
</tr>
<tr>
<td>Cortex</td>
<td>85 ± 7</td>
<td>60 ± 5</td>
<td>279 ± 14</td>
<td>265 ± 20</td>
<td>157 ± 14</td>
</tr>
<tr>
<td>Striatum</td>
<td>83 ± 6</td>
<td>75 ± 8</td>
<td>274 ± 18</td>
<td>271 ± 17</td>
<td>163 ± 11</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>134 ± 12</td>
<td>133 ± 13</td>
<td>691 ± 114</td>
<td>764 ± 95</td>
<td>94 ± 6</td>
</tr>
</tbody>
</table>

Values indicate relative optical densities (x10<sup>-3</sup>). Although ligand binding differed among regions, we did not detect a statistically significant effect of genotype on ligand binding within cortex (visual, somatosensory, and parietal cortex), hippocampus (CA1 region) and striatum (N=8 each). The ligands were: D-[2,3-<sup>3</sup>H]Aspartic acid (GE Life Sciences, Piscataway, NJ) for excitatory amino acid transporters (EAAT); DL-[5-methyl-<sup>3</sup>H]-(AMPA) (Perkin-Elmer, Boston) for α-amino-3-Hydroxy-5-methylisoxazole-4-propionic acid (AMPA) ionotropic glutamate receptors; [<sup>3</sup>H]Kainic Acid for ionotropic glutamate kainic acid receptors; [<sup>3</sup>H]MK-801 (American Radiolabeled Chemicals, St-Louis, MO) for the N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptors; [<sup>3</sup>H]muscimol (Perkin-Elmer, Boston) for ionotropic GABA<sub>A</sub> receptors.
Supplemental Figure 1. Normal cerebrovascular anatomy in FHM1 mutant mice.

Representative ventral (A, C) and dorsal (B, D) views of representative WT and S218L HOM brains show the circle of Willis anatomy, and pial arterial anastomoses between middle and anterior cerebral arteries, respectively, after transcardiac ink perfusion (1.4 ml, 0.5 ml/sec) under deep isoflurane anesthesia. Circles on the ventral surface (A, C) indicate where arterial diameters were measured, whereas circles on the dorsal surface (B, D) indicate the pial anastomoses (inset) that have been analyzed for their number and distance to midline. None of these endpoints significantly differed between WT and S218L HOM mice (Supplemental Table 3). Pial anastomoses were defined as the narrowest part of the vessel or half way between the nearest branch points of the anterior and the middle cerebral artery, localized by tracing the peripheral branches of these major vessels. ACA, anterior cerebral artery; MCA, middle cerebral artery; ICA, internal carotid artery; PCA, posterior cerebral artery; PComA, posterior communicating artery; BA, basilar artery.
Supplemental Figure 2. Increased mortality after stroke in FHM1 mutant mice.

Survival curves show the timing of mortality after filament middle cerebral artery occlusion (fMCAO). Mortality rate was (a) 100% and 0% within 24h after 60 min fMCAO in male S218L HOM and WT mice, respectively (n=5 and 4), and (b) 62% and 30% within 2 weeks after 30 min fMCAO in female S218L HET and WT mice, respectively (n=8 and 7).
Supplemental Figure 3. Normal density of Glutamate and GABA<sub>A</sub> binding sites in FHM1 mutant mice.

Representative autoradiograms on sagittal brain sections from WT and R192Q HOM mice showing specific binding for five commonly used ligands for ionotropic glutamate (N-methyl-D-aspartate, NMDA; α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate, AMPA; kainic acid, KA) and GABA<sub>A</sub> receptors and glutamate reuptake (EAAT) sites. Darker areas represent brain regions with higher levels of bound radioligand. Nonspecific binding, also shown, was not significantly different from background levels. Quantitative data are shown in Supplemental Table 4.
Movie 1. Cerebral blood flow changes during distal MCAO in WT mice.

This representative movie of CBF shows the spatiotemporal hemodynamic changes upon distal MCAO by a microvascular clip through a temporal burr hole. Imaging field was positioned as shown in Figures 3 and 4 and imaging was performed through intact skull. Color bar shows CBF as % of pre-ischemic baseline. Time after imaging onset is shown on the top. MCA is clipped between 1 and 2 min (clip artifact visible in the lower right of the imaging field). Approximately 1-2 min after clipping, a wave of further decrease in perfusion originates from ischemic core and spreads throughout the ipsilateral hemisphere; this wave has previously been shown to correspond to anoxic depolarization. During the 60 min dMCAO, 1 distinct wave of spreading hypoperfusion is spontaneously triggered in the WT; this hypoperfusion transient corresponds to a peri-infarct depolarization. Reperfusion is achieved by removing the clip approximately 60 min after the onset of ischemia.
Movie 2. Cerebral blood flow changes during distal MCAO in S218L HOM mutant mice.

This representative movie of CBF shows the spatiotemporal hemodynamic changes upon distal MCAO by a microvascular clip through a temporal burr hole, in S218L HOM mutant mice. Please see legend to Movie 1 for imaging details. During the 60 min dMCAO, 8 distinct waves of spreading hypoperfusion events are spontaneously triggered in the S218L HOM mutant; these hypoperfusion transients correspond to peri-infarct depolarizations and cause a lasting decrease in tissue perfusion in their wake.