

## Genomics and Therapeutic Advances

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Migraine has a strong genetic component. Causative genes have not yet been identified, except for familial hemiplegic migraine (FHM), a rare monogenic subtype of migraine with aura.<sup>1</sup> FHM is genetically heterogeneous: missense mutations in CACNA1A and SCNA1A, the genes encoding the pore-forming  $\alpha 1$  subunits of the neuronal voltage-gated  $\text{Ca}^{2+}$  channel Cav2.1 (or P/Q-type) and  $\text{Na}^+$  channel  $\text{Na}_v 1.1$ , respectively, cause FHM type 1 (FHM1)<sup>2</sup> and 3 (FHM3)<sup>3</sup>; mutations in ATP1A2, the gene encoding the  $\text{Na}^+$ - $\text{K}^+$  ATPase  $\alpha 2$  subunit, cause FHM type 2 (FHM2).<sup>4</sup> Additional FHM genes certainly exist and remain to be identified.<sup>5</sup>

FHM1 mutations cause functional changes in the Cav2.1 channel gene. P/Q-type  $\text{Ca}^{2+}$  channels are located in somatodendritic membranes and in presynaptic terminals throughout the brain, where they play a dominant role in initiating fast synaptic transmission at most central synapses.<sup>6</sup> For example, they produce gain-of-function changes in human recombinant Cav2.1 channels, mainly by shifting channel activation to more negative voltages and increasing of the open probability and single-channel  $\text{Ca}^{2+}$  influx.<sup>7-9</sup> In addition, knockin (KI) mice carrying FHM1 mutations (R192Q or S218L) show an increased P/Q-type  $\text{Ca}^{2+}$  current in cerebellar and cortical pyramidal neurons.<sup>10-12</sup> KI mouse models also show gain-of-function of cortical excitatory synaptic transmission due to increased action potential-evoked  $\text{Ca}^{2+}$  influx and probability of glutamate release at pyramidal cell synapses.<sup>12,13</sup> Moreover, cortical excitatory neurotransmission in FHM1 KI mice is less susceptible to G-protein coupled presynaptic inhibition by GABAB receptors activation; as a consequence, excitatory synaptic transmission is further facilitated in the presence of baclofen.<sup>13</sup>

The severe S218L mutation produces a larger gain-of-function of  $\text{Ca}^{2+}$  influx and glutamate release at cortical synapses than the mild R192Q mutation.<sup>13</sup> The induction and the propagation of cortical spreading depression (CSD) are facilitated in FHM1 KI mice,<sup>10-12</sup> and S218L KI mice have a lower triggering threshold for CSD and a faster rate of CSD propagation than R192Q KI mice. Moreover, the S218L mutation, but not the R192Q mutation, makes the cortex more susceptible to multiple successive CSD events in response to a single threshold stimulus.<sup>11</sup>

The more pronounced gain-of-function effects of the S218L mutation on cortical excitatory synaptic transmission and CSD correlate with the more severe S218L clinical phenotype, which may consist of (in addition to attacks of hemiplegic migraine) epileptic seizures, coma, and severe cerebral edema often triggered by only a trivial head

trauma.<sup>14</sup> These findings suggest that the gain-of-function effects of FHM1 mutations on cortical excitatory synaptic transmission and CSD may be involved in migraine pathogenesis.

To determine if gain-of-function mutations help to induce and propagate experimental CSD in FHM1 KI mice, we measured the threshold for CSD induction and the velocity of CSD propagation in acute slices of somatosensory cortex of R 192Q KI mice before and after perfusion with a concentration of the *P/Q* Ca<sup>2+</sup> 18-channel inhibitor ωAgalVA that reduced glutamate release at KI pyramidal cell synapses to wild-type (WT) levels. Strikingly, restoration of glutamate release to WT levels completely rescued CSD facilitation, as both CSD triggering threshold and CSD propagation rate in mutant mice became similar to those in WT mice.<sup>12</sup> This finding provides direct evidence of a causative link between enhanced glutamate release at pyramidal cell synapses due to gain-of-function of mutant *P/Q* Ca<sup>2+</sup> channels and facilitation of experimental CSD. On the whole, our findings support a model of CSD initiation in which release of glutamate from recurrent pyramidal cell synapses and activation of NMDA receptors are key components of the positive feedback cycle that overwhelms the regulatory mechanisms controlling the interstitial concentration of K<sup>+</sup> ions and ignites CSD. Indeed, we have recently found that saturating concentrations of either ωAgalVA or the NMDA receptor inhibitor AP5 prevent the induction of CSD in acute slices of sensory cortex of WT mice (with depolarizing stimuli up to 4 times larger than the control triggering threshold).

Our current work is aimed at testing the hypothesis that FHM1 mutations affect differently synaptic transmission at different cortical synapses and, as a consequence, produce functional alterations in the neuronal circuits that coordinate and dynamically adjust the balance between excitation and inhibition during cortical activity; these functional alterations may, in certain conditions (eg, during intense, long-lasting sensory stimulation or other migraine triggering states) lead to overexcitation and hyperactivity of cortical circuits, and as a consequence, render the cortex vulnerable to CSD ignition and explain the episodic onset of seemingly "spontaneous" CSDs; we further hypothesize that these alterations may underlie the abnormal processing of sensory information that characterizes the brain of migraineurs in the periods between migraine attacks.<sup>15,16</sup>

As a first test of this hypothesis, we investigated excitatory and inhibitory neurotransmission at connected pairs of layer 2/3 pyramidal cells (Pyr) and multipolar fast-spiking (FS) interneurons in acute slices of the somatosensory cortex of WT and R192Q KI mice, using paired patch-clamp recordings. Unlike at Pyr-FS synapses, inhibitory neurotransmission at FS interneuron synapses was unaltered in FHM1 mice.<sup>12</sup> More recently, we have investigated the total excitatory and inhibitory synaptic drive onto layer 2/3 pyramidal cells in acute cortical slices of WT and R 192Q mice, by measuring spontaneous excitatory and inhibitory postsynaptic currents in the presence of ongoing network activity. The spontaneous uncorrelated excitatory synaptic charge was larger in the mutant mice, as expected if the FHM1 mutation increases evoked glutamate release at recurrent pyramidal cell synapses. In striking contrast, the spontaneous uncorrelated inhibitory synaptic charge was similar in WT and KI mice.

Since, most likely, different types of inhibitory interneurons contribute to the measured inhibitory synaptic charge; this finding supports the conclusion that FHM1 mutations do not affect evoked GABA release at other types of inhibitory synapses in addition to those of FS interneurons. Our findings demonstrate that FHM1 mutations affect differently synaptic transmission at excitatory and inhibitory cortical synapses and, as a consequence, alter the neuronal circuits that dynamically adjust the balance between excitation and inhibition during cortical activity. We will discuss how these alterations may produce over excitation in certain brain conditions, but might leave the excitation-inhibition balance within physiological limits in others, thus explaining the episodic nature of the disease.

Our analysis of the functional consequences of FHM1 mutations give strong support to the view of migraine as an episodic disorder of brain excitability, with episodic disruptions of the excitation-inhibition balance and hyperactivity of cortical circuits in response to specific migraine triggers as the basis for episodic vulnerability to CSD ignition. Our data point to excessive excitatory synaptic transmission at recurrent cortical pyramidal cell synapses, due to both enhanced Cav2.1 dependent-dependent glutamate release and reduced presynaptic inhibition during G-protein coupled neuromodulation, as the basis for possible hyperactivity of cortical circuits and increased susceptibility to CSD in FHM1. Of course, several different molecular and cellular mechanisms may potentially lead to disruption of the excitation-inhibition balance and to increased vulnerability to CSD ignition in response to specific migraine triggers. This concept may explain the remarkable genetic and clinical heterogeneity of migraine. For the types of migraine in which the different molecular mechanisms converge to produce hyperactive cortical circuits and facilitate CSD induction (as probably occurs in the different types of FHM), agents that increase the trigger threshold for CSD are attractive candidates for novel migraine prophylactics. More in general, the view of migraine as an episodic disorder of brain excitability may be consistent with non converging parallel mechanisms leading to episodic disruptions of the excitation-inhibition balance in different (perhaps multiple) brain regions in response to specific migraine triggers. In this case, knowledge of the different molecular and cellular mechanisms would seem crucial for the development of novel migraine prophylactics tailored to distinct therapeutic targets in different patients.

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