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# $Ca_V2.1$ voltage activated calcium channels and synaptic transmission in familial hemiplegic migraine pathogenesis

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ABSTRACT

Studies on the genetic forms of epilepsy, chronic pain, and migraine caused by mutations in ion channels have given crucial insights into the molecular mechanisms, pathogenesis, and therapeutic approaches to complex neurological disorders. In this review we focus on the role of mutated $Ca_V2.1$ (i.e., P/Q-type) voltage-activated $Ca^{2+}$ channels, and on the ultimate consequences that mutations causing familial hemiplegic migraine type-1 (FHM1) have in neurotransmitter release. Transgenic mice harboring the human pathogenic FHM1 mutation R192Q or S218L (KI) have been used as models to study neurotransmission at several central and peripheral synapses. FHM1 KI mice are a powerful tool to explore presynaptic regulation associated with expression of $Ca_V2.1$ channels. Mutated $Ca_V2.1$ channels activate at more hyperpolarizing potentials and lead to a gain-of-function in synaptic transmission. This gain-of-function might underlie alterations in the excitatory/ inhibitory balance of synaptic transmission, favoring a persistent state of hyperexcitability in cortical neurons that would increase the susceptibility for cortical spreading depression (CSD), a mechanism believed to initiate the attacks of migraine with aura.



## 1. Migraine and Familial Hemiplegic Migraine (FHM)

Migraine is a common, chronic neurovascular disorder, typically characterized by recurrent attacks (1–3 days) of disabling headaches with associated autonomic symptoms. Twelve percent of the general population has on average one to two migraine attacks per month and treatments are frequently unsatisfactory. The etiology of migraine is multifactorial (for reviews, see Marmura and Silberstein, 2011; Goadsby et al., 2002; Pietrobon and Striessnig, 2003). The migraine pain is likely to be triggered by activation of the trigeminovascular system, which primarily modulate sensory signal transmission trough the activation of trigeminal afferents to meningeal blood vessels, the trigeminal nerve, and brainstem nuclei.

In 20% of cases, the migraine headache is preceded by a visual hallucination/illusion known as *aura*. There is growing evidence from animal models suggesting that CSD is the electrophysiological event underlying migraine aura (Haerter et al., 2005; Lauritzen, 1994). In experimental animals, CSD is an intense and steady depolarization of neuronal and glial cell membranes that last for less than one minute, which can spread to contiguous cortical areas of the brain at a rate of 3–5 mm/min, regardless of functional cortical divisions or arterial territories. Evoked when local extracellular $K^+$ concentrations exceed a critical threshold, CSD is associated with disruption of membrane ionic gradients, massive influxes of $Ca^{2+}$ and $Na^+$, and massive $K^+$ efflux with concomitant glutamate release. All such ionic unbalance is believed to depolarize adjacent neurons and glia, thereby facilitating its spread. An advancing wave of brief excitation would then be followed by a longer-lasting inhibition of spontaneous and evoked neuronal activity that traverses the cortex (Somjen, 2001; Goadsby, 2007; Pietrobon, 2005a,b; Lauritzen, 1994; Cutrer et al., 1998; Hadjikhani et al., 2001; Bowyer et al., 2001). In humans, evidence for a causal role of CSD in the aura comes from functional magnetic resonance imaging (fMRI) performed during migraine attacks with aura (Hadjikhani et al., 2001).

The nature and mechanisms of the primary brain dysfunction leading to the activation of the meningeal trigeminal nociceptors remain incompletely understood (for reviews see Charles, 2009; Goadsby et al., 2009a; Levy et al., 2009) and it is controversial whether CSD can initiate the migraine headache cascade by itself. Animal studies support the idea that CSD may also initiate the headache mechanisms, but the connection between CSD and headache in patients (particularly those with migraine without aura) remains an open question (Ayata, 2009; Pietrobon and Striessnig, 2003). On the other hand, there is experimental animal evidence that CSD might activate the trigeminal sensory system, presumably by depolarizing perivascular trigeminal terminals at meningeal and dural blood vessels (Haerter et al., 2005; Ayata et al., 2006;

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Bolay et al., 2002). Zhang et al. (2010, 2011) demonstrated, for the first time, that the induction of CSD by focal stimulation *in vivo* of the rat visual cortex can lead to long-lasting activation of the nociceptors innervating the meninges in the trigeminal ganglion.

Current views of migraine describe it primarily as a multifactorial brain disorder with an estimated heritability as high as 50% (Mulder et al., 2003). Genetic research in the field of migraines has mainly focused on the identification of genes involved in familial hemiplegic migraine (FHM), a rare monogenic subtype of migraines with aura. Genes for three monogenic subtypes of migraine have been identified so far (van den Maagdenberg et al., 2007): *CACNA1A* (FHM1, encoding the pore-forming $\alpha_1$ subunit of $Ca_V2.1$ calcium channels; Ophoff et al., 1996), *ATP1A2* (FHM2, encoding the $\alpha_2$-subunit of sodium–potassium ($Na^+$,$K^+$) pumps present in glial cell; De Fusco et al., 2003), and *SCN1A* (FHM3, encoding the pore-forming $\alpha_1$-subunit of neuronal $Na_V1.1$ voltage gated sodium channels, Dichgans et al., 2005).

More recently, in some patients suffering from migraine an homozygous mutation in the SLC4A4 gene (NBCe1, encoding the electrogenic Na(+)-HCO(3)(-) co-transporter was found; Suzuki et al., 2010). The immunohistological and functional analyses of these mutants demonstrate that the nearly total loss of NBCe1 activity in astrocytes can cause migraine potentially through dysregulation of synaptic pH.

A link between a common form of migraine and a genetic mutation of the TWIK-related spinal cord (TRESK) two-pore domain potassium channel (K2P), encoded by KCNK18, was recently reported (Lafreniere et al., 2010). By screening the KCNK18 gene in a large multigenerational family with typical migraine with aura, inherited in a dominant fashion, Lafreniere et al. (2010) identified a mutation in TRESK (F139WfsX24). They also identified prominent TRESK expression in migraine salient areas such as the trigeminal ganglion. The F139WfsX24 mutation produces a frame shift that prematurely truncates the channel in the second transmembrane domain and causes a complete loss of TRESK function. In addition, the mutant subunit suppresses wild-type channel function through a dominant negative effect, thus, explaining the dominant penetrance of this allele. It is expected that the TRESK mutation would make the trigeminal neurons more easily excitable. Increasing TRESK activity might help to diminish the excitability of these neurons, and may lessen migraine severity or incidence, whereas decreasing activity (through *KCNK18* mutations) may increase the risk of migraine.

A recent genome-wide association study in a large clinic-based sample of European individuals with migraine identified a genetic variant on chromosome 8q22.1 associated with migraine (Anttila et al., 2011). Furthermore, in a new study including 5122 migraineurs and 18,108 non-migraineurs, several single nucleotide polymorphism associations (SPNs) specific for migraine were identified (Chasman et al., 2011) establishing a link of migraine with LRP1 (modulator of glutamate signaling) and TRPM8 receptors (related to neuropathic pain models).

## 2. FHM type 2: Glial cells have a central role in migraine

Mutations in the *ATP1A2* gene are responsible for at least 20% of FHM cases. The FHM2 ATP1A2 gene encodes the $\alpha_2$ subunit of a $Na^+$, $K^+$ pump ATPase (De Fusco et al., 2003; Marconi et al., 2003). This catalytic subunit binds $Na^+$, $K^+$, and ATP, and utilizes ATP hydrolysis to extrude $Na^+$ ions out of the cell while moving $K^+$ ions in. $Na^+$ pumping provides the steep $Na^+$ gradient essential for the transport of glutamate and $Ca^{2+}$. The gene is predominantly expressed in neurons at the neonatal stage and in glial cells at the adult (De Fusco et al., 2003; Vanmolkot et al., 2003). An important function of this specific ATPase in adults is to modulate the key reuptake of potassium and glutamate from the synaptic cleft into the glia (Moskowitz et al., 2004). When this mechanism fails, it leads to elevated extracellular levels of glutamate and potassium, and accordingly, to an increased susceptibility to CSD (Pietrobon, 2007; Koenderink et al., 2005). All FHM2 mutations studied in heterologous expression systems result in a "loss-of-function" or a kinetically altered $Na^+$, $K^+$ pump (Pietrobon, 2007; Tavraz et al., 2008; Vanmolkot et al., 2006; De Fusco et al., 2003; Segall et al., 2005).

Leo et al. (2011) have reported the generation of the first mouse model of FHM2, a knock-in mutant harboring the human W887R-ATP1A2 mutation which decreases induction threshold and increases the velocity of propagation of CSD. The authors suggested a relatively minor role of the glial $\alpha_2$ Na, K pump in $K^+$ clearance meaning that the reduced CSD threshold in FHM2 knockin mice would not be primarily due to impaired $K^+$ reuptake. Instead their evidence point to an inefficient astrocyte clearance of glutamate and a consequent increased cortical excitatory neurotransmission.

## 3. FHM type 3: sodium channels and migraine

FHM type 3 is caused by missense mutations in SCN1A, the genes encoding the pore-forming subunits of the neuronal voltage-gated $Na^+$ channel $Na_V1.1$ (Dichgans et al., 2005).

Kahlig et al. (2008) investigated the functional consequences of two mutations linked to FHM3: Q1489 K and L263 V. Q1489 K mutation, when compared to WT-$Na_V1.1$, causes a predominantly loss-of-function phenotype, as deduced from a more rapid onset of slow inactivation, delayed recovery from fast and slow inactivation and a greater loss of channel availability during repetitive stimulation. However, opposite effects of the same mutation on tsA-201 cells and rat cultured neurons transfected with human $Na_V1.1$ were observed by Cestèle et al. (2008). L263 V exhibited biophysical abnormalities compatible with a gain-of-function mutation: increased persistent (not inactivating) current, delayed entry into fast and slow inactivation, depolarizing shifts in the steady-state voltage dependence of both fast and slow inactivation, accelerated recovery from fast inactivation, and greater channel availability during repetitive stimulation Kahlig et al. (2008). Notably, greater channel availability during repetitive stimulation, as seen for L263 V mutation, has also been observed for various epilepsy-associated SCN1A mutations (Rhodes et al., 2005) and might correlate with the unusually high prevalence of epilepsy observed in L263 V mutation carriers. The coincidence of migraine with epilepsy suggests that these two neurological disorders may share common mechanisms in L263 V mutation carriers.

$Na_V1.1$ is the major target for epileptogenic mutations and different mutations in this gene are known to be associated with epilepsy and febrile seizures (Meisler and Kearney, 2005; Avanzini et al., 2007). However, interestingly, just few cases of seizures have been reported in FHM patients (Dichgans et al., 2005; Vanmolkot et al., 2007). Thus, FHM mutations could be modifying $Na_V1.1$ differently compared to purely epileptogenic mutations.

Studies using heterologous expression and functional analysis of recombinant $Na_V1.1$ channels suggest that epilepsy mutations in $Na_V1.1$ may cause either gain-of-function or loss-of-function effects that are consistent with either increased or decreased neuronal excitability (Ragsdale, 2008; Cestèle et al., 2008). However, most $Na_V1.1$ mutations have their ultimate epileptogenic effects by reducing $Na_V1.1$-mediated whole cell sodium currents in GABAergic neurons, resulting in widespread loss of brain inhibition, an ideal background for the genesis of epileptic seizures (for review: Catterall et al., 2010; Ragsdale, 2008).

GABAergic interneurons are particularly sensitive to the loss of $Na_V1.1$ in gene-targeted mice (Yu et al., 2006; Ogiwara et al., 2007),