Recurrent Stroke Due to a Novel Voltage Sensor Mutation in Ca$_{\text{v}}$2.1 Responds to Verapamil

Ellen Knierim, MD; Lilia Leisle; Christiane Wagner, MD; Bernhard Weschke, MD; Barbara Lucke; Georg Bohner, MD; Jens P. Dreier, MD; Markus Schuelke, MD

**Background and Purpose**—Familial hemiplegic migraine is characterized by recurrent migraine, hemiparesis, and ataxia. Causes may be mutations in calcium and sodium channels or in a subunit of the Na/K-ATPse. Migraine treatment with calcium channel blockers was only successful in some patients.

**Summary of Case**—We describe a 6-year-old girl with recurrent ischemic strokes after minor head trauma associated with seizures, hemiparesis, fever, and altered consciousness. Genetic analysis revealed a spontaneous, novel dominant CACNA1A mutation (c.4046G$\rightarrow$A, p.R1349Q) that removed a highly conserved arginine of the voltage sensing region of the P/Q-type Ca$_{\text{v}}$.2.1 channel. Because a homologous mutation in the tottering-5J mouse increased open probability of the channel as well as calcium influx, we treated the patient with the calcium channel blocker verapamil during characteristic prodromi after head trauma. Treatment was instantly effective and prevented a new stroke.

**Conclusion**—CACNA1A mutations should be considered in the diagnostic workup of childhood stroke, especially if associated with ataxia and migraine. *(Stroke. 2011;42:e14-e17.)*

**Key Words:** calcium channels ■ genetics ■ pediatric neurology ■ pediatric stroke ■ stroke in children
studies, no obvious cause was found. Sequence analysis of all mitochondrial tRNAs ruled out mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome and related disorders. The girl recovered slowly, needed long-term rehabilitation, and a left-sided hemiplegia remained. Absence-like seizures were treated with levetiracetam. At 5 years of age, after falling from the lap of a grandparent, she was again admitted to the hospital with loss of consciousness, now with fever, right-sided focal seizures, and weakness. Serial MRI scans revealed increased signal intensity on T2 and fluid-attenuated inversion recovery images and hypointensity in apparent diffusion coefficient maps of the left hemisphere that were not restricted to vascular territories and showed variance in the temporal progression of the lesions (Figure 1C–H). Digital subtraction angiography excluded an arterial or venous occlusion. Over the next 3 months, vigilance and motor capabilities of the child improved slowly.

Genetics

The clinical symptoms and the MRI diagnostic findings led us to suspect a severe form of FHM and prompted sequence analysis of CACNA1A. Genomic DNA was isolated from peripheral blood cells by salt extraction and all 47 exons of CACNA1A (GenBank NM_001127222), including flanking intronic regions, were polymerase chain reaction-amplified and analyzed by automatic sequencing. We identified a heterozygous c.4046G→A transition in CACNA1A (Figure 2. Molecular genetic findings in the patient’s family. A, The heterozygous c.4046G→A transition leads to the exchange of arginine for glutamine and was absent in both parents. B, Segregation analysis of the mutation. The c.4046G→A mutation generates a novel BstN I restriction site, thus cleaving the 241-bp wild-type fragment into 171+70 bp (mut).)
paternity, we investigated 20 microsatellite markers flanking the uniparental disomy or a complex rearrangement and to confirm alleles of normal control subjects (data not shown). To exclude novo mutation was absent in her parents (Figure 2) and in 200 the substitution of arginine to glutamine at codon 1349. The de mutation (Figure 2B). On the protein level, the mutation causes

Shortly after we had found the CACNA1A mutation, at the age of 5.5 years, the girl was again admitted to the emergency department after another backward fall from a chair and hitting her head on the floor. She showed the characteristic prodromi that had anticipated her last ischemic strokes (all secondary to a minor head trauma), which comprised severe headaches, yawning, truncal unsteadiness, and progressive loss of consciousness. Based on the molecular genetic results, we decided to initiate immediately an intravenous therapy with the calcium channel blocker verapamil at a single dose of 0.1 mg/kg body weight over 20 minutes. Already during infusion the girl regained consciousness and started to talk to her mother. The dramatic clinical improvement was sustained even after the infusion had finished. As a long-term prophylactic treatment, we prescribed oral verapamil at a daily dosage of 3 mg/kg body weight under which the child did not develop any signs of stroke for 1 year despite 2 episodes of head trauma of the same order of magnitude as the ones causing stroke before.

Discussion

We report on a young girl who had several ischemic strokes due to a spontaneous dominant mutation in her CACNA1A gene (c.4046G→A, p.R1349Q). Ischemic stroke has not been reported in FHM1 before. These findings expand the clinical spectrum of CACNA1A mutations and place our patient at one extreme of a clinical continuum between simple migraine aura and the most severe vascular complications with manifest stroke.

The CACNA1A gene encodes the α1-subunit of the highly conserved P/Q-type voltage-gated calcium channel Ca_{2.1}. Analysis of the c.4046G→A sequence variant with Mutation Taster, an evaluation tool of the pathogenic potential of sequence variants, predicted pathogenicity with a high probability (R=0.988). Moreover, the absence of this variant in 200 normal alleles excluded a common polymorphism. The mutation replaces an evolutionary highly conserved alkaline arginine by a neutral glutamine. R1349 contributes to a motif of 4 arginines that form the voltage sensor of various calcium channels (ii) as well as sodium and potassium channels (iii). Note the complete conservation of the quadruple-arginine motif (*). The mutated amino acid is highlighted.

![Figure 3. Multiple sequence alignment of a critical sequence motif from the highly evolutionary conserved voltage sensing domain of CACNA1A orthologs from various species (i), of several voltage-gated fast and slow calcium channels (ii) as well as sodium and potassium channels (iii). Note the complete conservation of the quadruple-arginine motif (*). The mutated amino acid is highlighted.](http://stroke.ahajournals.org/)

Clinical Course and Verapamil Treatment

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Missense mutations removing such arginine residues have been shown to alter Ca_{2.1} channel function in a gain-of-function manner. This was confirmed by in vitro and ex vivo studies on human CACNA1A FHM1 mutations (p.R192Q and p.R583Q) and for the p.R1252Q mutation of the tottering-5j (Tg-5J) mouse (this mutation corresponds to p.R1300Q on Tg-5J) mouse is exactly homologous to the human p.R1349Q mutation (Figure 3). The functional consequences of this mutation were grave, because homozygous mice died soon after birth. We thus have reason to assume that in humans, the p.R1349Q mutation may also lead to a gain-of-function with enhanced Ca_{2.1} channel activity and subsequent increased intracellular calcium concentrations leading to cortical spreading depression.
Spreading depression of cortical electric activity is thought to be the pathophysiologic correlate of migraine aura and the result of near-complete and sustained depolarization of neurons above the inactivation threshold for their action-potential generating ion channels. Spreading depolarization can be observed as a large change of the slow potential, whereas spreading depression is characterized by electric silence in the higher frequency band of the electrocorticogram in humans and animals. Resistance vessels change their vascular tone in response to spreading depolarization, causing either (1) transient hyperperfusion (physiological hemodynamic response) in healthy tissue, or (2) severe hypoperfusion (inverse hemodynamic response) under pathological conditions leading to spreading ischemia, which in itself may cause widespread cerebral infarctions in animals and which contributes to lesion progression in humans. Mild head trauma is a well-known trigger for such spreading depolarizations, and missense mutations in CACNA1A may decrease their threshold, an effect presumably due to increased glutamatergic neurotransmission. The findings in our patient suggest that the inverse hemodynamic response to spreading depolarization might be promoted or mediated by Ca2+1 channels. Because Ca2+1 channels also seem to be present on the smooth muscle layer of resistance vessels, they may mediate vasoconstriction secondary to calcium influx. Interestingly, another l-type calcium channel blocker, nimodipine, was able to almost completely reverse spreading ischemia in a rat model.

We therefore hypothesize that the mild head trauma in our patient was sufficient to induce spreading depolarization, which, on top of an inverse hemodynamic response, led to widespread brain infarctions in a superadditive manner. This hypothesis deserves further study in animals, for example, in the tottering-5j (Tg-5J) mouse with the homologous mutation. We are aware that analysis of a single case cannot prove the therapeutic efficacy of verapamil in this condition. Further controlled clinical studies in combination with molecular genetic analyses are thus necessary to substantiate such an effect. Beyond that, our findings suggest that sequence analysis of CACNA1A should be considered during diagnostic workup of childhood stroke associated with migraine and ataxia; all the more, it seems to be a treatable condition.

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Disclosures
None.

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