

STIFF GOATS, CHLORIDE IONS, AND IDIOPATHIC GENERALIZED EPILEPSY (IGE)

Mutations in *CLCN2* Encoding a Voltage-Gated Chloride Channel Are Associated with Idiopathic Generalized Epilepsies

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Idiopathic generalized epilepsy (IGE) is an inherited neurologic disorder affecting about 0.4% of the world's population. Mutations in 10 genes causing distinct forms of idiopathic epilepsy have been identified so far, but the genetic basis of many IGE subtypes is still unknown. Here we report a gene associated with the four most common IGE subtypes: childhood and juvenile absence epilepsy (CAE and JAE), juvenile myoclonic epilepsy (JME), and epilepsy with grand mal seizures on awakening (EGMA). We identified three different heterozygous mutations in the chloride-channel gene *CLCN2* in three unrelated families with IGE. These mutations result in (a) a premature stop codon (M200fsX231), (b) an atypical splicing (del74–117), and (c) a single amino-acid substitution (G715E). All mutations produce functional alterations that provide distinct explanations for their pathogenic phenotypes. M200fsX231 and del74–117 cause a loss of function of ClC-2 channels and are expected to lower the transmembrane chloride gradient essential for γ -aminobutyric acid (GABA)ergic inhibition. G715E alters voltage-dependent gating, which may cause membrane depolarization and hyperexcitability.

are due to mutations in *CLCN1*—the gene that encodes the muscle voltage-gated chloride channel, which is a dimer of two pore-forming proteins (2,3). Now Haug et al. describe the first cases of mutations in a homologous gene family member expressed in human brain, *CLCN2*. Not unexpectedly, epilepsy is the salient phenotype. The affected individuals, each heterozygous for the chloride-channel mutation, were identified in three human pedigrees with IGE.

A closer look at the three gene mutations reveals that each is linked to a different channel defect and a reasonably distinct seizure phenotype. The first mutation leads to a premature stop codon, resulting in a truncated protein, loss of current, and a clinical syndrome of juvenile myoclonic epilepsy (JME) and epilepsy with grand mal seizures on awakening (EGMA). The second is a splicing mutation, which also produces a nonfunctioning channel and leads to childhood absence epilepsy (CAE). CAE has both a similar, but not identical, absence seizure and a generalized convulsive phenotype. The third is a missense mutation, yielding a channel that conducts current with altered kinetics, and a few patients in this pedigree display a less severe juvenile pattern of absence epilepsy with the delayed onset in a few cases of convulsive seizures. It is important to note that this multicenter study involved the participation of experienced neurologists following standard diagnostic classification criteria.

How does this welcome new addition to the list of epileptic channelopathies fit with our current understanding? Like other channel defects associated with epilepsy, mutations in *CLCN2* are capable of producing repetitive firing in neurons. But why does the *CLCN2* mutation result in seizures, and not spasticity, tremor, or episodic ataxia? Are interneurons not equally affected by the hyperexcitability? What are the extracellular or intracellular conditions that favor episodic symptoms appearing at specific ages? Do these triggers disappear in the individuals of pedigree 2 who became seizure free? Finally, what are the mechanisms underlying the dramatically different seizure phenotypes in these individuals?

Perhaps the answers to these questions will be found in detailed analysis of regional variations in the contribution of *CLCN2* to the firing patterns in different neural circuits at various developmental stages. For instance, in neonatal hippocampus, some expressed isoforms of *CLCN2* are truncated and functionally silent (4). In addition, it is important to

COMMENTARY

Since Adrian and Bryant first described the impaired chloride conductance of muscle in myotonic goats, it has been known that inherited disorders of membrane repolarization lead to hyperexcitability and repetitive firing in nerve and muscle (1). Both the recessive and dominant forms of myotonia congenita

consider the influence of other genetic differences among these three pedigrees, which potentially could modify the relative susceptibility for specific epilepsy syndromes among affected individuals. The importance of both examples is highlighted by the observation that mice lacking the *CLCN2* gene develop retinal deficiency but do not display epilepsy at all (5). Evidently, in the case of chloride anions, researchers must begin analysis in mutant mice by looking on the negative side.

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References

1. Adrian RH, Bryant SH. On the repetitive discharge in myotonic muscle fibres. *J Physiol* 1974;240:505–515.
2. Koch MC, Steinmeyer K, Lorenz C, Ricker K, Wolf F, Otto M, Zoll B, Lehmann-Horn F, Grzeschik KH, Jentsch TJ. The skeletal muscle chloride channel in dominant and recessive human myotonia. *Science* 1992;257:797–800.
3. Jentsch TJ, Stein V, Weinreich F, Zdebik AA. Molecular structure and physiological function of chloride channels. *Physiol Rev* 2002;82:503–568.
4. Mladinic M, Becchetti A, Didelon F, Bradbury A, Cherubini E. Low expression of the ClC-2 chloride channel during postnatal development: a mechanism for the paradoxical depolarizing action of GABA and glycine in the hippocampus. *Proc R Soc Lond B Biol Sci* 1999;266:1207–1213.
5. Bosl MR, Stein V, Hubner C, Zdebik AA, Jordt SE, Mukhopadhyay AK, Davidoff MS, Holstein AF, Jentsch TJ. Male germ cells and photoreceptors, both dependent on close cell-cell interactions, degenerate upon ClC-2 Cl⁻ channel disruption. *EMBO J* 2001;20:1289–1299.