

IDENTIFICATION OF A NEW JME GENE IMPLICATES REDUCED APOPTOTIC NEURONAL DEATH AS A MECHANISM OF EPILEPTOGENESIS

Mutations in *EFHC1* Cause Juvenile Myoclonic Epilepsy

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Juvenile myoclonic epilepsy (JME) is the most frequent cause of hereditary grand mal seizures. We previously mapped and narrowed a region associated with JME on chromosome 6p12–p11 (*EJM1*). Here, we describe a new gene in this region, *EFHC1*, which encodes a protein with an EF-hand motif. Mutation analyses identified five missense mutations in *EFHC1* that cosegregated with epilepsy or EEG polyspike–wave in affected members of six unrelated families with JME and did not occur in 382 control individuals. Overexpression of *EFHC1* in mouse hip-

podampal primary culture neurons induced apoptosis that was significantly lowered by the mutations. Apoptosis was specifically suppressed by SNX-482, an antagonist of R-type voltage-dependent Ca^{2+} channel ($Ca_v2.3$). *EFHC1* and $Ca_v2.3$ immunomaterials overlapped in mouse brain, and *EFHC1* coimmunoprecipitated with the $Ca_v2.3$ C terminus. In patch-clamp analysis, *EFHC1* specifically increased R-type Ca^{2+} currents that were reversed by the mutations associated with JME.

COMMENTARY

Juvenile myoclonic epilepsy (JME) is a relatively common form of generalized epilepsy, which displays a complex inheritance pattern. Mutations in ion channel genes have been associated with JME and include the calcium channel subunit *CACNB4* (1), the γ -aminobutyric acid (GABA) receptor subunit, *GABRA1* (2), and the chloride channel *CLCN2* (3). For each of these genes, mutations have been reported in only a single family with JME. Therefore mutations in ion channel genes are a rare cause of JME. Linkage studies combining numerous small families with JME have identified three additional loci: *EJM1* on chromosome 6p12–p11, *EJM2* on chromosome 15q14, and *EJM3* on chromosome 6p21. To date, no causative gene has been identified from the *EJM2* locus. The initially reported link between JME and the human lymphocyte antigen (HLA) region on chromosome 6p21 has now been designated *EJM3*, and association with *BRD2* was recently described (4,5). Suzuki and colleagues have now uncovered the gene responsible for JME in families mapping to the *EJM1* locus.

The *EJM1* locus was refined to a 3.5-centimorgan region containing only 18 genes. Mutation analysis of these genes revealed five different mutations in the *EFHC1* gene, in six Mexican families with JME. All mutations resulted in single amino acid substitutions. Two families had the same doubly heterozygous mutation and shared a common haplotype sur-

rounding the *EFHC1* gene, suggesting the two mutations arose on a founder chromosome common to both families. Mutations were found in six of 31 Mexican families and none of 12 European American families with JME. One large family from Belize carried a common polymorphism that segregated with JME. This polymorphism did not have any effect on normal *EFHC1* function in the experiments described later, supporting the hypothesis that this family is linked to *EFHC1* and that another, nearby mutation may be responsible for JME in this family. The fact that *EFHC1* mutations were not found in all families mapping to the *EJM1* locus implies that mutations may exist in noncoding regions that were not examined. Although splice sites and the upstream promoter region were sequenced, mutations may exist in control elements in other noncoding regions of the *EFHC1* gene.

The *EFHC1* protein is so named because it has an EF-hand domain, which functions as a calcium-binding motif. In mice, the *EFHC1* protein is localized to the soma and dendrites of pyramidal neurons in the cortex and Purkinje cells in the cerebellum. In cultured mouse neurons transfected with wild-type *EFHC1*, cells had shorter neurites and fewer branches. After 2 days in culture, the cells began to shrink and die and were positive for markers of apoptosis. Cell death was reduced by the addition of any of the five *EFHC1* mutants. Therefore the normal function of *EFHC1* appears to induce apoptosis, and the

JME mutations disrupt this complex process of programmed cell death.

Apoptosis involves genetic reprogramming of the cell to promote a cascade of biochemical and morphologic changes that result in cell death and elimination. Apoptosis can be triggered by many different stimuli, including calcium influx through plasma membrane channels. The presence of the calcium-sensing EF-hand motif prompted the authors to investigate whether EFHC1-induced apoptosis was associated with calcium channel activity. When calcium channels were blocked in neurons expressing EFHC1, cell-survival rate increased, implying that EFHC1 induces apoptosis through activation of calcium channels. Increased cell survival was seen only when R-type calcium channels (Ca_v2.3) were blocked. Blocking L-, T- or P/Q-type calcium channels had no effect on cell survival. With patch-clamp analyses in hamster kidney cells expressing Ca_v2.3, the authors demonstrated that EFHC1 greatly increased R-type calcium currents, providing further evidence that EFHC1 induces apoptosis specifically through activation of R-type calcium channels. *EFHC1* is expressed in several brain regions that overlap with Ca_v2.3 expression. In the hippocampus, both Ca_v2.3 and *EFHC1* are expressed in the soma and dendrites, implying that EFHC1 may bind directly to the calcium channel. With coimmunoprecipitation techniques, the authors confirmed that EFHC1 can directly interact with Ca_v2.3. They found that DM10 domains of EFHC1 bound to the C-terminus of Ca_v2.3 (and not the N- or P/Q-type channel subunits Ca_v 2.1 and Ca_v 2.2). In JME families, mutations are located within the DM10 domain region of EFHC1 shown to be involved in binding to Ca_v2.3, implying that the mutations disrupt binding. To summarize, the data suggest that mutations in *EFHC1* interrupt normal apoptosis by preventing the EFHC1 protein from binding to and activating R-type calcium channels.

How can reducing cell death lead to seizures? During development, apoptosis is necessary for tissues and organs to acquire their unique structures and functions. Growth of tissue is determined by a quantitative relation between the rate of cell proliferation and the rate of cell death. The authors suggest that mutations in *EFHC1* may cause unwanted neurons to be retained, resulting in increased density of neurons. Quantitative magnetic resonance imaging (MRI) has detected increases in cortical grey matter in JME patients, which may be due to an increase in the number of neurons (6). The increased density of neurons may cause JME by producing hyperexcitable circuits as a result of altered neuronal connectivity.

Polymorphisms in *BRD2* have been associated with a clinically identical JME syndrome (4). *BRD2* may also be involved in apoptosis, because of its proposed function as a mitogen-activated protein (MAP) kinase. MAP kinases are important mediators of signal transduction and play a key role in the

regulation of many cellular processes, such as cell growth and proliferation, differentiation, and apoptosis. *LGII*, the gene responsible for partial epilepsy with auditory features, also has been implicated in MAP kinase signaling (7). Valproate (VPA) is a commonly used and effective treatment for JME. It is interesting that VPA targets calcium channels (8) and also indirectly regulates a number of factors involved in cell-survival pathways (9), including MAP kinases. Mutations in cystatin B that cause PME of the Unverricht-Lundborg type also are associated with defects in apoptosis (10) However, this severe form of myoclonic epilepsy has been associated with increased neuronal cell death rather than increased cell survival.

An emerging role appears for several epilepsy genes, such as *EFHC1*, *CSTB*, *BRD2*, and *LGII*, in the development and maintenance of normal neuronal structures, through cell-survival and cell-death pathways. Subtle increases in specific populations of neurons may be associated with JME, whereas extensive cell death is associated with more severe, progressive myoclonic epilepsy. Regardless of whether cell numbers are increased or decreased, it would disrupt the normal balance between excitatory and inhibitory circuits. Therefore a common epilepsy mechanism may involve abnormalities in functional connectivity that are due to subtle structural changes in the brain.

by Robyn Wallace, Ph.D.

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