

JUVENILE MYOCLONIC EPILEPSY: IS IT AN IDIOPATHIC EPILEPSY CAUSED BY A MALFORMATION OF CORTICAL DEVELOPMENT?

EFHC1 Interacts with Microtubules to Regulate Cell Division and Cortical Development. de Nijs L, Léon C, Nguyen L, Loturco JJ, Delgado-Escueta AV, Grisar T, Lakaye B. *Nat Neurosci* 2009;12(10):1266–1274. Mutations in the *EFHC1* gene are linked to juvenile myoclonic epilepsy (JME), one of the most frequent forms of idiopathic generalized epilepsies. JME is associated with subtle alterations of cortical and subcortical architecture, but the underlying pathological mechanism remains unknown. We found that EFHC1 is a microtubule-associated protein involved in the regulation of cell division. *In vitro*, EFHC1 loss of function disrupted mitotic spindle organization, impaired M-phase progression, induced microtubule bundling, and increased apoptosis. EFHC1 impairment in the rat developing neocortex by *ex vivo* and *in utero* electroporation caused a marked disruption of radial migration. We found that this effect was a result of cortical progenitors failing to exit the cell cycle and defects in the radial glia scaffold organization and in the locomotion of postmitotic neurons. Therefore, we propose that EFHC1 is a regulator of cell division and neuronal migration during cortical development and that disruption of its functions leads to JME.

COMMENTARY

The concept of idiopathic epilepsies has undergone dramatic revisions in recent years. According to its Greek origins, *idios* means “one’s own” or “pertaining to one’s self,” implying that an idiopathic disease represents a primary, isolated disorder without any other cause or associated diseases. Alternatively, the word *idiopathic* is often used simply to mean “of unknown cause.” Recent insights into the molecular genet-

ics and pathophysiology of idiopathic epilepsies have threatened to make the term “idiopathic” obsolete.

Ever since idiopathic epilepsy syndromes were described, based on characteristic clinical and electroencephalographic features, a genetic etiology was suspected because of occasional familial groupings. Over the past decade, a number of genes have been identified as causing various idiopathic epilepsies, especially in cases with defined familial patterns of inheritance. Most of these genes encode for ion channels, such as voltage-gated sodium or potassium channels, or GABA-receptor channels. Thus, these disorders fall into an emerging group, classified as channelopathies. Although most identified genes causing

idiopathic epilepsy syndromes encode ion channels, pathogenic genes have not yet been found in the majority of cases of idiopathic epilepsies, especially sporadic cases. Furthermore, a few genes have been discovered that produce nonchannel proteins.

Juvenile myoclonic epilepsy (JME) represents one of the most common types of idiopathic epilepsy. JME is a generalized epilepsy, classically characterized by onset during adolescence, the presence of myoclonic seizures, variable frequency of generalized tonic-clonic and absence seizures, and a 4–6 Hz generalized spike- or polyspike-wave pattern on EEG. Although JME is one of the prototypical idiopathic epilepsies, detailed pathological and radiographic studies have previously suggested that subtle anatomical abnormalities may contribute to the pathophysiology of this disease. In particular, postmortem pathological analysis of the brains of presumed JME patients demonstrate dysmorphic neurons and subtle abnormalities in cortical architecture, consistent with microdysgenesis (1). Furthermore, quantitative MRI has found increases in cortical gray matter volume in JME patients (2,3). Consistent with the initial discovery of genes causing other idiopathic epilepsies, the first couple of genes associated with JME involved mutations in genes for ion channels, such as the GABA_A receptor (4). Shortly thereafter, however, mutations in a novel, nonchannel gene, *EFHC1*, were identified as the cause of some cases of familial JME (5). Subsequent studies suggest that *EFHC1* mutations are currently the most commonly identified cause of JME, although most cases of JME still have no identified cause (6).

Myoclonin 1, or EF-hand domain containing protein 1 (EFHC1), is a nonchannel protein with an EF-hand domain possessing calcium-binding functions. Initial studies of EFHC1 function indicated that this protein promotes apoptosis by activation of R-type calcium channels. Mutations in the *EFHC1* gene inhibited apoptosis, suggesting that excessive neuronal survival and density in cortex could account for structural abnormalities in JME (5). Interestingly, other studies reported that EFHC1 is a homolog of an axonemal protein found in motile cilia and flagella (7). While these novel findings suggested that EFHC1 might interact with microtubule-based cytoskeletal elements, another study found that EFHC1 was specifically associated with mitotic spindles during cell division in cultured cell lines (8). Overall, these observations raise the tantalizing possibility that *EFHC1* gene mutations might cause disruptions in cell division and survival during early corticogenesis, with resultant developmental brain abnormalities leading to epilepsy in affected JME patients.

The recent study by de Nijs et al. more directly addresses the potential role of EFHC1 in cortical development. Extending previous work documenting the association of EFHC1 with mitotic spindles (7), the investigators first demonstrated that EFHC1 represents a novel microtubule-associated protein, or

MAP, that directly binds α -tubulin and is required for normal mitotic spindle organization in HEK293-cultured cells in vitro. Furthermore, in potential conflict with a previous study (5), loss of function of EFHC1 caused an increase, not decrease, in apoptosis. Most impressively, the authors extended the functional analysis of EFHC1 to the brain in situ, utilizing technically demanding ex vivo and in utero electroporation of EFHC1 short hairpin RNA (shRNA) to inactivate EFHC1 function in the developing rat brain. With these methods, they demonstrated that focal inactivation of EFHC1 disrupts several processes of normal embryonic cortical development. EFHC1 inactivation interfered with mitosis and cell cycle exit of cortical progenitors in the ventricular zone. Furthermore, the normal radial migration of progenitor cells and projection neurons out of the ventricular zone, the organization of radial glia scaffolding, and subsequent locomotion of postmitotic neurons to the cortical plate were all impaired by EFHC1 inactivation.

Overall, these novel findings indicate that *EFHC1* mutations may interfere with multiple steps in embryonic cortical development. These effects are most likely primarily related to a disruption of normal interactions of EFHC1 with mitotic spindles, causing an arrest of cortical progenitor cells in the ventricular zone and subsequent downstream inhibition of neuronal migration. In addition, migration of postmitotic neurons might also be impaired as a result of direct effects of the dysfunctional microtubule-associated EFHC1 protein on microtubule-based locomotion. Thus, this study supports the intriguing idea that an idiopathic epilepsy—JME—might actually be caused by a subtle malformation of cortical development.

Although there is some merit and previous data to support this paradigm-shifting idea of JME as a type of cortical malformation (1–3), there also are several possible discrepancies and paradoxes among different studies on EFHC1 function. First, while the present study concluded that EFHC1 inactivation leads to *increased* apoptosis, a previous study found the opposite (5). A decrease, not increase, in apoptosis might be more consistent with the radiographic findings of increased cortical gray matter volume in JME patients (2,3). Second, a recently created *Efch1*^{-/-} null mouse exhibits increased seizure susceptibility, enlarged ventricles, and reduced hippocampal size (9). However, no grossly apparent structural abnormalities of neocortex were reported in these mice, in contrast to what might be predicted by the impaired mechanisms of cortical development demonstrated in the present study. Similarly, the reported effects of EFHC1 inactivation on progenitor cell turnover and neuronal migration might be expected to lead to a dramatic cortical phenotype in humans, such as lissencephaly or double-cortex resulting from *LIS1* or *DCX/DCLK* mutations, which also have microtubule-associated protein-like functions. However, the available neuropathological studies of potential relevance to JME, reveal much more subtle cortical abnormalities,

consisting of microdysgenesis (1). Finally, as *EFHC1* mutations probably account for only a minority of cases of all JME (10), other pathophysiological mechanisms likely also are important in generating the phenotype of JME. Thus, while the present study is exciting and provocative in supporting a novel pathophysiology of JME, additional mechanistic studies of *EFHC1* function as well as detailed clinicopathological studies of JME patients are needed to determine whether idiopathic JME is actually caused by a malformation of cortical development.

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