

Juvenile myoclonic epilepsy: *EFHC1* at the cross-roads ?

Epilepsy is a complex disease, accounting for up to 1% in the population, and is characterized by recurrent seizures.^{1,2} The primary cause for epilepsy could be either genetic or environmental. The former group comprises both polygenic forms and monogenic forms. The heritable Mendelian monogenic forms are, however, rarer traits.^{1,2} A majority of the genetic forms of epilepsy – both Mendelian and non-Mendelian forms, are classified as “idiopathic epilepsy”, a group characterized by the absence of detectable form for brain lesions.^{1,2} The primary symptom of epilepsy, the epileptic seizure, could either be provoked by specific sensory stimulus (for example flash, stress or sound) or could be unprovoked and the phenotype could range from subtle changes in the behaviour (absence seizure) to violent jerks (myoclonic seizures).^{1,3} Since all form of seizures result from abnormal electrical discharges in the neurons, and since neuronal electrical output is regulated by ion channels, defects in genes coding for ion channel proteins have long been thought to be the genetic cause for the idiopathic forms of epilepsy.⁴ Therefore, epilepsy syndromes are often grouped under “channelopathies”.^{4,5} Consistent with this view, there has been an upsurge, in the last one decade, in the discovery of epilepsy-causing mutations in genes encoding for ion channel proteins.⁶ While a considerable number of genes that code for channel proteins have been identified to be defective in epileptic patients, the number of independent families that carried these mutations were very few and hence such forms are considered as rarer epilepsies.^{2,7} One of the exceptions to the school of “channelopathy” is the discovery of mutations in the *EFHC1* gene in a significant number of families with juvenile myoclonic epilepsy (JME).^{8,11} JME is one of the most common epilepsy syndromes accounting for 7% of all cases of epilepsy.^{2,12} The seizures in JME may begin between late childhood and early adulthood, and is characterized by myoclonic seizures usually in the early morning, soon after awakening.^{2,12} JME

patients may also exhibit tonic-clonic seizures and absence seizures.^{2,12}

The JME gene *EFHC1* is ubiquitously expressed and codes for an EF-hand-containing calcium binding protein named as myoclonin.^{8,13} Two splice variants for the *EFHC1* gene, resulting from differential splicing of exon 4, have been identified.⁸ The protein isoforms coded by these variants differ in their carboxyl terminal; the protein coded by the minor isoform is expected to lack the EF-hand although the functional significance of this transcript is not known.⁸ Intriguingly, JME associated mutations have been identified for both the transcripts.^{8,11} While missense mutations have been identified for the protein coded by the major transcript, a deletion/frameshift mutation was reported for the minor transcript. JME is thought to manifest as autosomal dominant disorder,⁸ and consistent with this view all the mutations that are reported so far in *EFHC1* were found to show heterozygosity in the affected individuals.⁸ However whether or not the disease phenotype results from a gain or loss of function of the mutant protein is yet to be clearly established. Moreover, whether or not proteins coded by both the splice variants are involved in the etiology is yet to be tested. Nonetheless, functional studies on the *EFHC1* protein, using in vitro and in vivo models, have uncovered rather unexpected physiological functions for this protein and have led to the proposal of three contrasting and apparently exclusive models for the epileptogenesis (see Fig. 1). This commentary highlights the recent findings on the *EFHC1* gene functions and discusses its relevance to the genesis of epilepsy.

EFHC1 as a pro-apoptotic protein: removing the unwanted?

The *EFHC1* gene was discovered in the JME critical region 6p12-p11 in the year 2004 and its protein product was found to localize in the soma and dendrites on the neurons.^{8,14} Ectopic overexpression of *EFHC1* resulted in shorter neurites and a

fewer branches and the neurons showed signs of degeneration and eventually underwent apoptotic form of cell death.⁸ Intriguingly, the overexpression of JME associated *EFHC1* mutant proteins did not lead to such abnormal neuronal features, suggesting that the mutations might have affected some of the functional properties of *EFHC1*; one of such functions could be positively regulating the apoptotic process.^{7,8} The *EFHC1* protein harbors an EF-hand domain and this domain has been shown to bind to calcium.^{8,13} It is known that binding of calcium induces a conformational change in the EF hand domain-containing proteins which, in most cases, result in the changes in the functional property of the target proteins that often catalyze an enzymatic reaction.¹⁵ Therefore, the authors tested whether the *EFHC1*-induced cell death observed in the neuronal cultures is mediated through the calcium channels. Overexpression of *EFHC1* in the presence of calcium channel blockers increased the survival rate of neurons, confirming the hypothesis that *EFHC1*-induced cell death is mediated through calcium signaling.⁸ Indeed *EFHC1* was found to positively regulate activity of calcium channels, and the increased influx of calcium ions could underlie the apoptosis.⁸ Thus, it could be concluded that the JME linked mutations in the *EFHC1* might affect the apoptotic process in the brain and that absence of neuronal death could underlie the epileptic symptoms in JME patients.^{7,8} This conclusion brings in an intriguing question; why should neurons be killed for a brain to be healthy? An answer to this question comes from our understanding of the developmental processes. Programmed cell death – the alternate name for apoptosis, is an important event during development of the embryo and also its central nervous system. Nearly 70% of the developing neurons are known to be eliminated by programmed cell death at various stages for the morphogenetic events like the neural tube closure.¹⁶ Thus loss of *EFHC1* might affect this process and brain may end up having additional and unwanted neurons in

unwanted places resulting in the hyperexcitable circuits and the epileptic seizures.⁷ This is the first of the three alternate hypothesis on the role of *EFHC1* in JME.

EFHC1 as an axonemal protein: beating for a cause?

The domain organization of *EFHC1* protein is almost identical to Rib72 – an axonemal protein of the unicellular flagellate, *Chlamydomonas*.¹⁷ The axoneme represents the inner cytoskeletal compartment of the flagellum or the cilium and provides support to its movement.¹⁷ The Rib72 of *Chlamydomonas* is thought to function in the structural assembly of the axoneme and in the regulation of flagellar motility.¹⁷ Intriguingly, the murine homologue of *EFHC1* was found to be abundantly localized to the sperm flagella and tracheal epithelial cilia – the cell types that are known to have axonemal cytoskeleton, suggesting that *EFHC1* might be required for the axonemal function in mammalian cells as well.^{18,19} However, the causal link between ciliary/flagellary function and epilepsy is yet to be established because ciliary/flagellary defects should lead to additional phenotypes (for example, poor fertility, respiratory problems, etc) and such symptoms are not commonly seen in JME patients. Is there a connection between the functions of the cilium and the brain? Yes, indeed; the brain harbors canal-like structure, known as ventricular system, and the cells that line the surface of the canal is characterized by the presence of long cilia. These cells, called the ependymal cells, help in the circulation of the cerebrospinal fluid around the central nervous system, through their rhythmic beating.^{20,21} Mutations affecting this ependymal flow of the cerebrospinal fluid might result in hydrocephalus – a developmental anomaly associated with the increased intracranial pressure and progressive enlargement of the head in humans.²¹ A recent study demonstrated that *EFHC1* is indeed expressed in ependymal cells, and is localized in cilia.²² While the ciliary structure was unaffected, the ciliary beating was severely impaired in the *EFHC1* null mice, suggesting that

EFHC1 is required for the normal functions of ependymal cells.²³ Intriguingly, the null mice also displayed spontaneous myoclonus and also showed increased susceptibility for induced seizures. Thus, reduction or loss of function of *EFHC1* protein results in impaired ciliary function in the ependymal cells and enhanced seizure susceptibility and epileptogenicity. This is the second alternate hypothesis for the role of *EFHC1* in JME.

EFHC1 and cell cycle: A neuronal navigator?

Cell biological investigations on the *EFHC1* protein led to some unexpected observations. Firstly, *EFHC1* was found to associate with the mitotic spindle, and the centrosome.²⁴ Since the subcellular localization of the protein varied during the mitotic cycle, and since only a fraction of the protein gets recruited to the mitotic apparatus, it was suggested that *EFHC1* could be a microtubule associated protein, and a potential role for *EFHC1* in mitotic cell division was envisaged.²⁴ The *EFHC1* gene is expressed maximally in the brain during its embryonic development – a stage where cell division is at maximum, suggesting that *EFHC1* could possibly be involved in cell cycle regulation in the developing brain.^{8,24} What should be the connection between cell cycle and JME? Emerging studies show that neuronal cytoskeletal machinery regulates the cell-cycle of the neuronal progenitors, and that the proliferation of the progenitor cells in the developing cortex is critical for proper neurogenesis, neuronal migration, and axonal wiring.^{25,26} Since these three events – neurogenesis, neuronal migration, and axonal wiring – are dynamic in

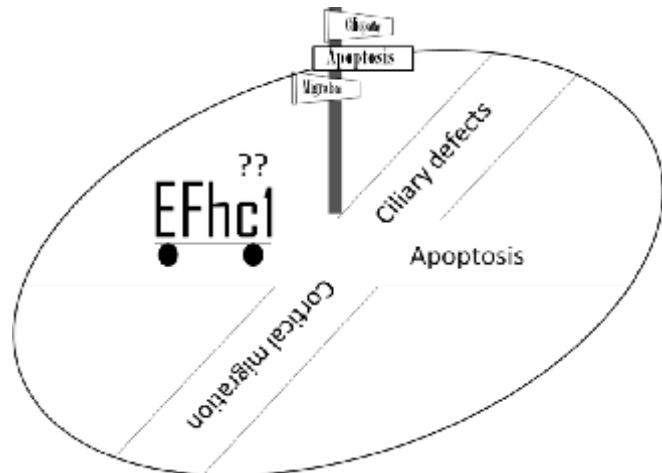


Fig. 1: Cartoon depicting the direction of three distinct working hypotheses on the role of *EFHC1* protein in the etiology of JME. The *EFHC1* protein implicated in the proapoptotic function, in the functions of the cilia and in the migration of the neurons. However which one of these three pathways is involved in the epileptogenesis is unclear.

nature, and are regulated by microtubule-associated proteins, a causal role for *EFHC1* in the proliferation of neuronal progenitors and migration was proposed and tested.²⁷ Firstly, it was shown that overexpression of truncated versions of the *EFHC1* in a human kidney cell line resulted in the impaired mitotic spindle defects, and chromosomes misalignments during the metaphase, and apoptosis.²⁷ Conversely, RNAi-mediated knockdown of *EFHC1* in the same cell line led to an increase in the mitotic index, suggesting that *EFHC1* negatively regulates M-phase progression.²⁷ Secondly, knockdown of *EFHC1* in developing cortical region severely affected the neuronal migration. This defect could possibly be due to the fact that the progenitor cells that were devoid of *EFHC1* did not exit the cell-cycle and continued to proliferate mitotically.²⁷ Taken together these results have demonstrated that *EFHC1* is a regulator of cell division and neuronal migration during development, and defects in this process may underlie some of the symptoms seen in JME. This is the third alternate hypothesis for the role of *EFHC1* in JME.

EFHC1, the epilepsy gene: where it leads us to?

The positional cloning of genes for monogenic neurological disorders has

thrown up a large number of previously uncharacterized genes into the mainstream of neuroscience research. In a way similar to the "reverse genetics" screen in model systems, the functional studies on genes associated with the diseases like JME have discovered novel links between common cellular processes and brain specific disorders. For *EFHC1*, for example, none of the three proposed mechanisms call for critical role for ion channel, an age old concept that epilepsy results from primary defects in the ion channels. The proposed functions of the *EFHC1* proteins – that (i) it promotes cell death, (ii) is involved in ciliary movement, and that (iii) it regulate the neuronal differentiation and their migration – may appear to be apparently unconnected. Nonetheless, each of these hypotheses propose the structural changes in brain to be an underlying cause for the epileptogenesis, and that the JME could be a developmental disorder that manifests in the adolescence. The emerging knowledge on *EFHC1* questions the very concept that the JME is one of the idiopathic epilepsies – that is epilepsy without structural lesions in the brain. With growing knowledge in brain development and function, and further studies on the *EFHC1* gene, the next few years should witness a significant improvement in our understanding on the cellular functions of *EFHC1* protein, and how defects in this process may lead to some of the symptoms seen in JME. Such strides might help us in developing effective strategies for early interventions and therapeutics.

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References :

- Turnbull J, Lohi H, Kearney JA. Sacred disease secrets revealed: the genetics of human epilepsy. *Hum Mol Genet.* 2005; 14: 2491-500.
- Delgado-Escueta AV, Perez-Gosienfiao KB, Bai D, *et al.* Recent developments in the quest for myoclonic epilepsy genes. *Epilepsia* 2003; 44: 13-26 (Suppl 11).
- Durón RM, Medina MT, Martínez-Juárez IE. Seizures of idiopathic generalized epilepsies. *Epilepsia* 2005; 46:34-47 (Suppl 9)
- Hahn A, Neubauer BA. Sodium and potassium channel dysfunctions in rare and common idiopathic epilepsy syndromes. *Brain Dev.* 2009; 31:515-520.
- George AL Jr. Inherited Channelopathies Associated with Epilepsy. *Epilepsy Curr* 2004; 4:65-70.
- Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. *Prog Neurobiol.* 2009; 87: 41-57.
- Ganesh S, Singh S. Neuronal survival in epilepsy: to die or not to die? *J Biosci* 2005; 30: 561-566.
- Suzuki T, Delgado-Escueta AV, Aguan K, *et al.* Mutations in *EFHC1* cause juvenile myoclonic epilepsy. *Nat Genet* 2004; 36:842-849.
- Stogmann E, Lichtner P, Baumgartner C, Idiopathic generalized epilepsy phenotypes associated with different *EFHC1* mutations. *Neurology* 2006; 67: 2029-2031.
- Annesi F, Gambardella A, Michelucci R, *et al.* Mutational analysis of *EFHC1* gene in Italian families with juvenile myoclonic epilepsy. *Epilepsia.* 2007; 48:1686-1690.
- Medina MT, Suzuki T, Alonso ME, *et al.* Novel mutations in *Myoclonin1/EFHC1* in sporadic and familial juvenile myoclonic epilepsy. *Neurology* 2008; 70: 2137-2144.
- Delgado-Escueta AV. Advances in genetics of juvenile myoclonic epilepsies. *Epilepsy Curr* 2007; 7:61-67.
- Murai MJ, Sassonia RC, Zamboni AH, *et al.* Characterization of the C-terminal half of human juvenile myoclonic epilepsy protein *EFHC1*: dimer formation blocks Ca²⁺ and Mg²⁺ binding to its functional EF-hand. *Arch Biochem Biophys* 2008;477: 131-138.
- Léon C, de Nijs L, Chanas G, *et al.* Distribution of *EFHC1* or *Myoclonin 1* in mouse neural structures. *Epilepsy Res* 2010; 88: 196-207.
- Grabarek Z. Structural basis for diversity of the EF-hand calcium-binding proteins. *J Mol Biol* 2006; 359: 509-525.
- De Zio D, Giunta L, Corvaro M, *et al.* Expanding roles of programmed cell death in mammalian neurodevelopment. *Semin Cell Dev Biol* 2005; 16: 281-294.
- Ikeda K, Brown JA, Yagi T, *et al.* Rib72, a conserved protein associated with the ribbon compartment of flagellar A-microtubules and potentially involved in the linkage between outer doublet microtubules. *J Biol Chem* 2003; 278: 7725-7734.
- Ikeda T, Ikeda K, Enomoto M, *et al.* The mouse ortholog of *EFHC1* implicated in juvenile myoclonic epilepsy is an axonemal protein widely conserved among organisms with motile cilia and flagella. *FEBS Lett.* 2005; 579: 819-822.
- King SM. Axonemal protofilament ribbons, DM10 domains, and the link to juvenile myoclonic epilepsy. *Cell Motil Cytoskeleton* 2006; 63: 245-253.
- Ibañez-Tallon I, Heintz N, Omran H. To beat or not to beat: roles of cilia in development and disease. *Hum Mol Genet* 2003;12: R27-35.
- Ibañez-Tallon I, Pagenstecher A, Fliegau M, *et al.* Dysfunction of axonemal dynein heavy chain *Mdnah5* inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation. *Hum Mol Genet* 2004; 13: 2133-2141.
- Suzuki T, Inoue I, Yamagata T, *et al.* Sequential expression of *Efhc1/myoclonin1* in choroid plexus and ependymal cell cilia. *Biochem Biophys Res Commun* 2008; 367: 226-233.
- Suzuki T, Miyamoto H, Nakahari T, *et al.* K. *Efhc1* deficiency causes spontaneous myoclonus and increased seizure susceptibility. *Hum Mol Genet* 2009; 18: 1099-10109.
- de Nijs L, Lakaye B, Coumans B, *et al.* *EFHC1*, a protein mutated in juvenile myoclonic epilepsy, associates with the mitotic spindle through its N-terminus. *Exp Cell Res* 2006; 312: 2872-2879.
- Shu T, Tseng HC, Sapir T, *et al.* Doublecortin-like kinase controls neurogenesis by regulating mitotic spindles and M phase progression. *Neuron.* 2006 Jan 5;49(1):25-39
- Weimer JM, Anton ES. Doubling up on microtubule stabilizers: synergistic functions of doublecortin-like kinase and doublecortin in the developing cerebral cortex. *Neuron* 2006; 49: 3-4.
- de Nijs L, Léon C, Nguyen L, *et al.* *EFHC1* interacts with microtubules to regulate cell division and cortical development. *Nat Neurosci* 2009; 12: 1266-1274.