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**Specific Aims**

Juvenile myoclonic epilepsy (JME) is responsible for 10-30% of epilepsy cases worldwide and is the most frequent cause of hereditary epilepsy [1]. The disease typically presents during puberty, beginning with myoclonic seizures, or muscle spasms, and progresses to full loss of consciousness seizures within a few years [2]. The genetic analysis of JME affected families led researchers to the association of the EFHC1 gene [3]. EFHC1 encodes a calcium binding protein, unusual for epilepsy [3]. EFHC1 is involved in apoptosis and mitosis, cellular events that require microtubules [4]. EFHC1 protein contains four domains: an EF hand calcium-binding domain and three DM10 domains [1]. The function of these repeating DM10 domains remains unknown [5]. *Without basic understanding of the functionality of EFCH1’s domains, our understanding of the causation of JME remains incredibly limited, and possible treatment options overlooked.*

***The primary goal of this research is to create a better understanding of the role of the EFHC1 protein in epilepsy***. I will specifically analyze the three DM10 domains within the EFHC1 protein. The pattern of three repeating DM10 domains within a protein only occurs in mammalian EFHC1 and chlamydomonas Rib72, a protein component of cilia in green algae that shares 40% sequence identity with human EFHC1 [6]. Rib72 binds microtubule structures and other coiled coil proteins to form protofilament ribbons needed for cilia movement [5]. Because of the similarity of sequence and domain structure of Rib72 and EFHC1 proteins, ***I hypothesize that EFHC1 protein binds cellular microtubules via DM10 domains.*** I will explore this hypothesis using three specific aims:

**Aim 1: To determine which DM10 domains in EFHC1 bind microtubules.**

**Approach:** Structure function analysis will be conducted using domain information gathered from SMART and PFAM databases. Each domain of mouse EFHC1 protein will be isolated and analyzed for its ability to bind to microtubules using microtubules tagged with a fluorescent tag.

**Hypothesis**: Each of the three EFHC1 DM10 domains will bind microtubules while the EF hand domain will be unable to bind microtubule units.

**Aim 2: To analyze conserved DM10 amino acids among EFHC1 homologs.**

**Approach:** Clustal Omega alignment program will be used to analyze and align protein sequence of human EFHC1 homologs. Specific amino acids found as point mutations in human epileptic studies will be analyzed throughout other homolog sequences.

**Hypothesis:** Amino acids implicated in epilepsy causation will be conserved throughout EFHC1 homologs, as they are vital for proper protein function.

**Aim 3: To find all the protein domains of EFHC1 interaction partners to observe which are involved in microtubule modification.**

**Approach:** EFHC1 interaction partner proteinswill be identified using STRING database and the domains analyzed for microtubule action using PFAM and SMART databases.

**Hypothesis:** Many EFHC1 interaction partners will have protein domains involved in modification of or binding to microtubules.

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# [2] Genton et. al. *Clinical Aspects of Juvenile Myoclonic Epilepsy.* Epilepsy and Behavior Journal. Volume 28. July 2013. <http://www.sciencedirect.com/science/article/pii/S152550501300005X>

# [3] Delgado-Escueta et. al. *The quest for Juvenile Myoclonic Epilepsy genes.* Epilepsy and Behavior Journal. Volume 28. July 2013. <http://www.sciencedirect.com/science/article/pii/S1525505012004957>

# [4] De Nijs et. al., *Juvenile myoclonic epilepsy as a possible neurodevelopmental disease: Role of EFHC1 or Myoclonin1*. Epilepsy and Behavior. Volume 28. July 2013. <http://www.sciencedirect.com/science/article/pii/S1525505012004969>

#  [5] King, Stephen. *Axonemal Protoﬁlament Ribbons, DM10 Domains, and the Link to Juvenile Myoclonic Epilepsy.* Cell Motility and the Cytoskeleton. Volume 63. 2006. <http://onlinelibrary.wiley.com/store/10.1002/cm.20129/asset/20129_ftp.pdf?v=1&t=hturpcy2&s=ac620ef3194976e78700d12e0d250a790d2c6f20>

# [6] Ikeda 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.* Journal of Biological Chemistry. November 2012. <http://www.jbc.org/content/278/9/7725.abstract>