Chapter-7

Summary and conclusions
SUMMARY AND CONCLUSIONS

BRD2 was the part of the Bromodomain and Extra-Terminal (BET) protein. The gene maps to the major histocompatibility complex (MHC) class II region on chromosome 6p21.3. BRD2 gene polymorphism could significantly promising therapeutic targets in the prevention of JME. We found six missense mutations (8%) and two non-sense mutations (2.6%) of BRD2 gene in unrelated JME patients. The direct sequencing of the BRD2 gene exhibited a heterozygous missense mutations in 7th exon changes the nucleotide bp c.3150G>A, c.8919G>A and c.3753G>C polymorphism. Exon 11 &12 revealed c.11832C>A, c.11648 A>T, c.11744G>A mutations and two non-sense mutations alters the c.9699 C>T and c.9827 A>T in 9th exon. One single nucleotide substitution found in LGI4 gene in exon 2-3. These results represent the highest number and percentage of mutations rate found for a JME disorder causing gene for any population group. Thus, it is important for mutation in JME cases suggested to undergo family molecular screening, because detection of BRD2 mutations provides an accurate diagnosis of JME risk.

Based on our results we suggest that BRD2 gene is more prone to mutations in JME than the other IGE. The hypothesis supported by our findings shows five pathological mutations (two had nonsense mutations and three had missense mutations). However, we must analyze more families of JME to provide the stronger evidence in support of this hypothesis. The effects of missense mutations at the protein level can give useful and interesting insight into the molecular basis of hereditary diseases. More comprehensive insights about mechanisms of pathogenicity of mutations, the analysis could be complemented with studies the effects of mutations at the DNA and RNA level.

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The molecular consequences of missense mutations should be viewed in the cellular context. A cellular level view of the mechanisms of pathogenicity could be achieved by applying a systems biology approach. In missense mutations gain clinical attention and usually change in the physicochemical properties of the amino acid residue sufficiently to affect the function of the gene product (Krawczak et al. 1998; Stone and Sidow 2005), but the most severe mutations are likely to result in lethal phenotypes that cannot be inherited (Steward et al. 2003).

Prediction of the molecular effects of disease causing missense mutations by bioinformatics methods has been implemented in numerous recent studies. Loss of protein stability leads to loss of enzymatic activity of hyper neuronal excitability and possibly to the accumulation of the protein in cells.

Missense mutations account for approximately half of all allelic variants underlying inherited human diseases (Hamosh et al. 2005; Krawczak et al. 2000; Stenson et al. 2003). Missense mutations exert more subtle effects on protein structure and function. The consequences of missense mutations can be more difficult to predict because of their diverseness and a single amino acid change may lead to multiple effects.

The present study showed that c.9699 C>T and c.9827 A>T polymorphism of nonsense mutations at base pair 254 and 896 in 9th exon of BRD2 gene associated with JME risk in Indian population. The functional effect of a nonsense mutation depends on the location of the stop codon within the coding DNA. A nonsense mutation can change in one DNA base pair and instead of substituting one amino acid for another; however, the altered DNA sequence prematurely signals the cell to stop building protein. This mutation enables the cell to insert an amino acid in response to the nonsense codon, resulting in a wild-type phenotype.
Nonsense mutations typically behave like loss-of-function alleles. Recent development indicates that BRD2 gene encodes a protein making channel at electrical synapse between neurons.

We identify the eight nonsynonymous single nucleotide polymorphisms (nsSNPs) in BRD2 gene that may contribute to epileptogenesis and genetically complex forms of the disease and cellular mechanisms that can promote neuronal hyper excitability. These allelic variants occur in the conserved exons, the consequence may be serious, results the pathogenic mutation. These exonic mutations important for JME risk families to undergo molecular screening of BRD2 gene and implications in the diagnosis, prognosis and genetic counseling of JME disorder.

In our case-control study reveals a new novel mutation, and nucleotide changes c.10497 G>N at 112 bp of LGI4 gene. According to the bioinformatics analysis it was not a pathogenic mutation, because allelic variants observed in only one JME patient. This kind of mutation not observed in control subjects. The absence of functional study of the mutation does not give any idea of its pathogenic effect. Hence the pathogenicity of sample c.10497 G>N mutation is still under discussion and functional studies are needed to further determine its pathogenicity of LGI4 gene. Another report reveals that exonic polymorphisms in LGI4 coding region (63) nucleotide changes c.975 C>T in exon 5, c.1353 G>C in exon 7, c.1722 A>G in exon 8, c.1914 GC>AT and c.2010 G>A in exon 9 were identified in childhood absence epilepsy (CAE) and benign familial infantile convulsions (BFIC).

In the animal model revealed that Lgi4 gene has been secreted from Schwann cells. Schwann cell is principal cellular source of LGI4 protein in the developing peripheral nerve tissue. All axons in the peripheral nervous system (PNS) are surrounded by Schwann cells and undergo a wrapping process.
Schwann cells signaling molecules are an important unanswered question. PCR technique of molecular analysis of nDNA and mtDNA was useful as a diagnostic tool in the identification of specific genetic traits or for the detection of pathogenic mutation. A better understanding of the molecular nature of JME in an individual was important to design a personalized medication, considering the number of possible genetic mutations that can contribute to genetic based JME.

Non-ion channel genes such as LGI4 gene has been contribute the myelin sheath increase speed of electrical impulses approximately two orders of magnitude faster than unmyelinated fibers of similar diameter. It gives insulates the axon and assembles voltage-gated sodium channel cluster at discrete nodes. Demyelination of sheath underscored by the large range of neurological diseases associated with its dysfunction. However, more studies are required to add to the pool of information and to help us to better understand the genetic structures of diverse in South Indian population groups, where many questions remain unanswered.

The exact mechanism of JME involved different gene mutations is still unknown. Further Familial and twin studies are required to investigate the strong involvement of LGI4 gene between the central nervous system (CNS) and peripheral nervous system (PNS).

The most common seizure-precipitation factor was sleep deprivation and low percentage of photosensitivity observed in the present study. A sleep EEG or EEG on awakening confirm the clinical suspicion of JME. Neuroimaging, MRI technique, suggest subtle structural and functional changes, mainly within the frontal lobes, in patient with JME. The majority of the JME patients will have associated with GTCS, which occur predominantly on walking or during sleep.
The patient at risk for GTCS increases morbidity, mortality and failure to diagnose the condition increases the risk of JME patient’s morbidity. Neurologic examination (30%-50%) and overall intelligence of JME patients must be normal by the neuroimaging. Specific learning disorders are permitted. Interictal EEG must show a normal background with bursts of generalized fast spike–wave (SW) or polyspike–wave. To make a correct diagnosis, clinicians need to ask specifically about myoclonic jerks.

Demands further research by means of advanced neuroimaging techniques using inter-ictal background EEG targeting structural aberrations in frontal lobe, lateral lobe and occipital lobe areas of the brain. Future large cohort studies should aim to establish genotype–phenotype correlations for mutations in the BRD2 gene to gain a better understanding of the molecular aetiology and to facilitate personalized treatment for JME.

The combined studies provided data on 229 polymorphisms in 55 different genes in different loci of chromosomes. Nevertheless, only six polymorphisms (three missense mutations and two nonsense mutations in BRD2 and one point mutation in LGI4 gene) in two genes have been associated with JME in, at least, two independent gene candidate investigations. The lack of success in replicating the results is related to various aspects, including limitations of experimental design, familial study, and genetic heterogeneity. Therefore, scientists should go beyond replication criteria. Such an integration of results from different experimental approaches combined with epigenetics and genomic technology could lead us to a more comprehensive evaluation of the current state of JME susceptibility.
An effective therapy for JME is not yet available and the currently used antiepileptic medications, suffer from unwanted side effects. Breakthrough in genetic engineering and gene editing technologies applications should hopeful lead to better therapy for JME disorder.

New bioinformatics methods that evaluate gene regulatory and splicing variants will broaden our understanding of functional variation. There is a need to increase in the field of molecular based genetic research and radio imaging studies will further aid in devising new treatments and cure for patients with JME. National and international collaborations and support from funding agencies is needed to conduct quality of research. Understanding the process of JME may identify the genetic markers for reorganization and interventions.
ABSTRACT

Background

Abnormal protein accumulation may be attributed to dysfunctional mitochondria and damage to the reactive oxygen species (ROS), leading to errors in calcium signaling to promote the seizures in juvenile myoclonic epilepsy patients. Mitochondrial mutations may exert unfavorable effects on neuron synapses which may lead to the loss of motor functions causing seizures. Mitochondrial disorders are a group of clinically heterogeneous diseases, commonly defined by a lack of cellular energy due to oxidative phosphorylation (OXPHOS) defects and ROS can cause somatic mutations in mtDNA. Currently, more than 250 pathogenic mtDNA mutations have been identified. Mitochondrial displacement loop (D-loop) is the hot spot for mtDNA alterations which influence the generation of cellular ROS. In the mtDNA there are areas that do not encode controller (D-loop) and repeat sequence undergo mutations at a very high rate ranging from 10^-6 to 10^-2 per generation. Such mutations are commonly caused by the loss of DNA mismatch repair (MMR). MtDNA mutations are involved with the mitochondria microsatellite instability (mtMSI), HV1 and HV2 segments, and may play a specific role in the genetic based epilepsy.

Although several proteins related with signaling, assembling, transporting, and enzymatic function can be impaired by the mitochondrial diseases, most frequently the activity of the respiratory chain protein complexes is primarily or secondarily affected, leading to impaired oxygen utilization and reduced energy production.

MtDNA D-loop region
Methods

The two hypervariable segments (HV1 and HV2) of mtDNA D-Loop region were sequenced in 25 JME patient’s blood samples. The Genomic DNA was extracted and amplified by polymerase Chain Reaction (PCR), and then SNPs were detected by direct sequencing and comparing the sequencing results with the mtDNA Cambridge Reference Sequence.

Result

We detected pyrimidine transitions 27% (HV1), 56% (HV2); purine transitions 23% (HV1), 39% (HV2) and transversion 50% (HV1), 5% HV2 segments in D loop region. However, overall we observed 64 mutations in two segments of HV1 and HV2 of D-loop region. We observed 29 copy number variation (CNV) in the form of microsatellite instability (MSI), Sequence mismatch (SMM), deletion and insertions in the two segments of mtDNA D-loop region.

Conclusion: We found several multiple mutations in D loop region, suggesting these mutations may be involved in the pathogenesis of JME as many neurological disorders are associated with mitochondrial mutations and mitochondrial dysfunction. Further studies including large scale of normal healthy individuals from the same ethnicity may help in identifying the role of these genetic variations in the pathogenesis of JME. Further investigations should focus on the tRNAs and rRNAs loci in the mtDNA mitochondrial genome to understand the mechanism of mtDNA and nDNA.

Keywords: mtDNA; Hypervariable region, MSI; D-loop; Juvenile myoclonic epilepsy, ROS