The size of the present case-control study population used in the present study is relatively standard sample; the findings are however for JME in the South Indian population. We found the SNPs tested showed a moderate but statistically significant association (phenotype frequency) with the JME. The allele could bring about subtle changes in the BRD2 gene function, and result in the JME seizures. However, in the present study among the twelve exons, nonsynonymous single nucleotide polymorphism (nsSNPs) detected in 7th, 9th exons (Missense mutations) and 11-12 exons (Nonsense mutations). Novel exonic variants displayed the highest frequency of missense mutations (8%) and two non-sense mutations (2.6%) were found in unrelated JME cases.

The direct sequencing of the BRD2 gene exhibited a heterozygous missense mutations c.3150G>A, c.8919G>A and c.3753G>C polymorphism. In exon7 and three missense SNP c.11832C>A, c.11648 A>T, c.11744G>A observed in 11th and 12th exons. Two non-sense mutations altered the base pair c.9699 C>T and c.9827 A>T in 9th exon. Our results had important implications in the diagnosis, prognosis and genetic counseling of JME phenotype in the present study. Nonsense mutations that generate termination codons in the coding region of a gene cause premature termination of protein synthesis. Nonsense mutations can be suppressed by mutant tRNAs that can read termination codons as sense codons, restoring the synthesis of an active gene product (34). Since nonsense mutations are associated with an increasing number of human genetic diseases (35). We found in our case-control study, overall frequency of polymorphic genotype in BRD2 gene to be 8.6% mutation rate. The present study showed that higher phenytoin levels more common in the adolescence and adult age groups. We also found that females had two times odds of developing JME related o males. No clear hot spot has been identified in the BRD2 gene locus.
6.1 Central nervous system associated with BRD2 (RING3) gene

In recent report investigate in animal model revealed that cerebral structure abnormalities connected to the motor and premotor areas of cerebral cortex, thalamus, red nucleus of midbrain and spinal cord. The Brd2 is playing important role in development of central nervous system (36). BRD2 has a putative nuclear transcriptional regulator gene, which plays an important function in the development of the central nervous system.

To understand the molecular mechanisms by which mis-expression of Brd2 might contribute to epilepsy, Brd2 protein only can be detected in the cerebellar Purkinje cells and not in hippocampal cells. These multiple levels of regulation would likely affect the production of functional BRD2 protein during neural development and hence, its role in JME susceptibility (Figure 5.1). The cerebral structural abnormalities during brain development in IGE patients may result in disorganized neuronal connectivity and regions of neocortical hyperexcitability, leading to clinical seizures.

The human BRD2 gene has been strongly linked and associated with JME risk and electroencephalographic abnormalities. The human BRD2 gene has been shown to play a critical susceptibility role in a common form of JME, neural phenomena including abnormal EEG patterns (37, 38). Critical to the potential regulation of BRD2 function has our discovery of an alternatively spliced exon within a highly conserved intronic or exonic region in both the human and mouse genes. The structural organization of the introns and exons of the human and mouse genes has been highly conserved. Epilepsy mutations affect proteins that regulate action potentials and synaptic function, both of which underlie neuronal communication. BRD2 deficient embryonic fibroblast cells were observed to proliferate more slowly than cells from controls, and enhanced levels of cell death in brd2 deficient embryos (Shang
et al., 2009). However, others reports disclosed the over expression of gene leads to neuronal degeneration suggesting positive regulation of apoptosis of neurons by BRD2 gene. In general during embryogenesis at the time of CNS development 70 to 80 % of neurons are subjected to apoptosis at various stages for morphogeni events. BRD2 is essential for chromatin structures and transcription during mammalian embryogenesis and neurogenisis.

The effect of BRD2 in single nucleotide polymorphism on promoter function is currently unknown, but they may alter the timing, tissue structure or level of expression (28). The BRD2 gene expresses distinct tissue-specific transcripts that originate from different promoters and have strikingly different lengths of 5’UTR. Neurodegeneration leads to the loss of anatomy and physiology of the nervous system. Most degenerative diseases is abnormal folding are accumulation of proteins within neuronal cell bodies, these changes in normal protein metabolism often lead to neuronal cell death and failure of affected regions of the central nervous system.

Figure 5.1 schematic diagrams showing the proposed function of BRD2
The proposed functions of the BRD2 proteins—(i) it promotes apoptosis, (ii) it regulates the purkinje cells differentiation (iii) it regulates synaptic functions and (iv) regulate the ataxia and nsSNPs in the protein structure might developed the JME risk.

6.1.1 Purkinje cells of cerebellar cortex associated with JME

The cerebellar cortex consists of three layers; external molecular layer, middle purkinje layer and internal granular layer. The purkinje dendrites extend into the molecular layer and axons synapse into deep cerebellar nucleus of granular layer. Axons from the inferior olive are called climbing fibers and a single climbing fiber axon makes hundreds of excitatory synapses on the purkinje neuron. The purkinje cells use GABA as a neurotransmitter, so their influence on cerebellar output is inhibitory.

Dysfunction of Purkinje cells can lead to lack of motor coordination (ataxia), a characteristic symptom of many debilitating movement disorders (Ito, 1984). Neuronal programmed cell death during embriogenesis and development of CNS has estimated 70% to 80% at various stages for the morphogenetic events. BRD2 mRNA was detected in human brain including cerebral cortex, cerebellum, medulla, occipital cortex, frontal cortex, putamen, brain vesicles, neural tube, spinal cord, dorsal root ganglion in the anatomical context of the gene concern. BRD2 is a putative developmental transcription regulator expressed in brain and may be involved in the JME cortical microdysgenesis (31).

Neuropathology of some patients with JME reveals increased number of and diffusely distributed dystopic neurons in gray matter stratum molecular and subcortical white matter of brains (27). In 2003, Pal et al. showed that SNPs within the BRD2 (RING3) gene present on chromosome 6p21.3 might serve
as susceptibility alleles (odds ratio 6.5) for autosomal recessive JME families from New York [31]. Three susceptibility mutant alleles have been associated with an increased risk of developing JME in connexin 36. Fourteen patients (18.6%) had a positive family history of epilepsy. The 61 JME (81.3%) patients met the criteria for classic JME in connexin36 gene [39]. The strong linkage disequilibrium with two JME SNP variants in the promoter region of BRD2 and a common variant haplotype in over 50% of 20 probands from families of European origin that had produced positive LOD scores for 6p21. BRD2 is essential for chromatin structures and transcription during mammalian embryogenesis and neurogenesis. Multi generational JME families that had originally been genetically linked to chromosome 6p21.3 (Human leukocyte antigen [HLA] region) and associated with HLA alleles, as in New York families of European origin. In the recent study by Pal and colleagues analyzed 20 single nucleotide polymorphism (SNPs) in 20 probands in the chromosomal loci on 6p21 region associated with JME and found strong genetic relations with eight SNPs in BRD2 gene.

BRD2 gene between alternative exon 2 and exon 3 were shows highly polymorphic microsatellite and allele was strongly associated with JME (Greenberg et al., 2000). In the present study we observed heterozygous nsSNPs in the form of missense mutations (6) in exon 7th and 11th & 12th, two nonsense mutations in 9th exon, but these nsSNPs not detected in the normal healthy controls. For the first time, proof for the emerging concept that JME has a complex mode of inheritance that was dependent on one or more disease genes and interplay of modifier genes was provided through this genetic research.
6.2 Molecular association in coding region of LGI4 gene polymorphism

Molecular screening analysis of LGI4 coding region in benign familial infantile convulsions (BFIC) and childhood absence epilepsy (CAE) noted several frequent exonic polymorphisms. A genotypic association was found for the c1914 GC>AT polymorphism in 42 CAE patients compared with 110 population controls. The predominant IGE subtype in the families supporting evidence for linkage was CAE. These linkage findings of LGI4 gene indicate a susceptibility gene for CAE close to the markers D19S414, D19S225.

6.2.1 Involvement of Lgi4 gene associated with regulation of myelination in animal model

The structural and functional maturation of myelinated nerve fibers in the PNS is governed by a temporally and spatially controlled series of molecular interactions between the axon and the Schwann cell (40). Schwann cells cover the axons in peripheral nervous system (PNS) with many layers of plasma membranes, containing lipids and proteins called myelin sheath. The myelin sheath act as an insulator along with nodes of Ranvier and volted-gated sodium channels are highly accumulated and generate action potentials (Figure 5.2). Schwann cell produces part of myelin sheath along a single axon of PNS neuron and participates in the regulation of PNS axon. Disorder in myelin sheath result in neurological diseases associated with its dysfunction. In claw paw mice and suggesting that Adam22 has a receptor for Lgi4 in the developing nerve (41). During the development of PNS myelinated nerve fibres of molecule called gliomedin, secreted from myelinated schwann cells. The genetic mutations that affect the glial cells, which damage the myelin sheath and process called demyelination. Leucine-rich glioma-inactivated (LGI) proteins and mutations in their genes have been associated with epilepsy and demyelization (Figure 5.3). LGI4 has multiple functions including proliferation of enteric glia and satellite cells in PNS, and later, myelin formation in schwann cells.
In animal model Lgi4 function has been primarily analyzed in the context of the developing PNS where it is secreted from Schwann cells and binds to ADAM22 in the axonal membrane (Nishino et al., 2010; Ozkaynak et al., 2010). The Lgi4 and Adam22 (A disintegrin and metalloprotease 22 protein) are both expressed in Schwann cells as well as in sensory neurons and that Lgi4 binds directly to Adam22 without a requirement for additional membrane associated factors. Schwann cells are the principal cellular source of Lgi4 in the developing nerve and Adam22 required on axons and sensory neurons also express Lgi4 at low levels (42). The interaction between Lgi4 and ADAM22 is required for timely axonal sorting and myelination (43).

In human model involvement of myelin sheath formation in PNS by Schwann cell, outside the myelin sheath, a thin layer of schwann cell cytoplasm persists to form an additional sheath has called neurilemmal sheath (Schwann cell sheath). Neurilemmal sheath may be important in directing the regeneration of axon to their appropriate targets (Figure 5.2). Myelin sheath increases the velocity of conduction of impulses and provides insulating sheath surrounding the fibres.
The Schwann cells act as signaling pathways, that controls axon segregation and myelin formation (42). The structural and functional mutation of myelinated nerve fibers in the peripheral nervous system (PNS) depends on the molecular interaction between the axon and Schwann cell (Jessen and Mirsky, 2005). The unique function of Lgi4 depends on small set of amino acids that present a novel interaction surface in these proteins. Myelin surrounds the axon of some nerve cells, forming an electrically insulating layer. It is essential for the proper functioning of the peripheral nervous system.

In inherited JME disorder, the myelin sheath does not develop properly, due to alter the single amino acid sequence in LGI4 gene and develop the seizures. Symptoms include progressive loss of thinking skills, and muscle weakness. Epileptic seizures are associated with LGI4 protein release from the subtle damaged regions of myelin sheaths during epileptic seizures, and the change of LGi4 protein in myelin sheath may play an important role in the pathogenesis of epilepsy.

Lgi4 protein implicated as a positive regulator of myelin formation in the PNS and polymorphism may reduce myelin formation in neuron-Schwann cell and causes hypomyelination in the peripheral nerve (Bermingham et al. 2006). Lgi4 gene has been expressed by neural crest stem cells but its expression restricted to the glial cells, which exist throughout the nervous system and include Schwann cells, astrocytes and oligodendrocytes (42). The neuronal axons and dendrites become coated with a segmented lipid-rich sheath (myelin) to enable faster and more energetically efficient conduction of electrical impulses.
Figure 5.3 Schematic diagram show the proposed function of LGI4 gene in Schwann cell and development of seizures

The myelin sheath formed by the cell membranes of Schwann cells in PNS (43, 44). The presence of an altered single amino acid change in a LGI4 protein reflects a point mutation. LGI4 gene by contrast, a mutation within an axon of a structural gene can alter the functions of the gene product and cause a phenotypic change. The mutations in the DNA germ line cells might be transmitted by gametes to the next generation (45, 46).
6.3 Precipitation of seizures in Juvenile myoclonic epilepsy

In our case control study sleep deprivation (35%) was more frequently reported by patients with JME. The other precipitation factors include awakening (24%), emotional stress (16%), menstruation (8%), T.V watching (5%) and other fatigue appear (12%). During sleeping, specialized neurons in the thalamus with profuse connections to the entire brain gradually disrupt the individual activities of cortical neurons and entrain them all into monotonous rhythmic synchronized discharges (47). Therefore, synchronized activity of large numbers of neurons abolishes their normal ‘wakeful’ functions.

Precipitation of seizures in patients with JME involved several factors includes sleep deprivation (54.2%), menstruation (20%), fatigue (9.2%), stress (7.6%), concentration (6.9%), photic stimulation (1.5%), TV/video (1.5%) (Murthy et al. 1998). In another study, fever and emotional disturbances were perceived as seizure precipitants in 29% and 16% of patients respectively (48). Sleep deprivation was found to be the most important precipitating factor in juvenile myoclonic epilepsy (54%) (49). Sleep deprivation was more frequently reported by patients who had also reported emotional stress. There was a strong correlation between sleep deprivation and stress. There has been some speculation that stress might lead to physiological changes in the corticosteroid levels as well as in the cerebral blood flow facilitating seizure occurrence (50). The EEG revealed a 4 to 6-Hertz polyspike and wave discharge, which in the younger child with absence seizures may be indistinguishable from that of typical absence epilepsy. In 10 to 15 percent of patients with JME, the initial EEG has normal (51, 52). About 15% of patients with JME report that flickering lights (disco, sunlight, and videogames) are provocative (53). Interictal activity revealed short bursts of polyspikes and polyspike-wave complexes after spontaneous or induced awakenings (54, 55). In general, sleep architecture affected, with decreased quality and sleep fragmentation.