INTRODUCTION

JME is a genetically determined idiopathic generalized epilepsy (IGE) syndrome and probably involves multiple genes due to widespread distribution in the central nervous system (CNS) and minor influence of environmental factors were contributed. The genetic back ground for JME account for 5-10% of all forms of epilepsy. About 7% of the siblings of probands with JME and in all type of epilepsy of which 30% will have JME. The age of onset is between 8 to 18 years with peak age at 13.5 to 15.5 years that affects both genders. Mutation within exons of a structural gene can alter the functioning of the gene product and cause a dramatic phenotypic change in amino acid sequence.

OBJECTIVES

1. To support innovative research, of the highest scientific merit, that has the greatest potential for patient benefit.
2. To identify the mutation of BRD2 gene in nuclear DNA in JME patients

METHODS: The case-control association study design was utilized to test the potential involvement of BRD2 gene variations in the etiology of JME. We performed molecular screening of BRD2 gene coding sequence for the detection of mutation through genomic PCR amplification and direct sequencing by ABI PRISM® 377 DNA Analyzer.

RESULT: These nonsense mutations involved exon 9 and most of them are autosomal dominant. Direct sequencing of the BRD2 gene showed a heterozygous nonsense mutation at nucleotides 254 and 896 base pairs of exon 9 in two affected unrelated JME cases.

INTERPRETATION & CONCLUSION: The exact genetic mechanism of JME is still unknown due its multiple genes involvement. Further Familial and twin studies are required to investigate the involvement of BRD1, BRD2 and BRD3 genes in the genetic susceptibility of JME syndrome in central nervous system.

KEYWORDS: BRD2 gene, JME, M.I, Nonsense mutation, SNP, WES

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ABSTRACT

Point Nonsense Mutations in BRD2 gene of Juvenile Myoclonic Epilepsy (JME) patients: A study from Dravidian Linguistic South Indian Population.
the BRD2 gene

Table: 1 Description of the oligonucleotides used for the analysis of BRD2 gene

<table>
<thead>
<tr>
<th>Primers name</th>
<th>Primer sequence</th>
<th>Amplified size (bp)</th>
<th>Annealed temperature (°C)</th>
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<tbody>
<tr>
<td>BRD2/E1</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
</tr>
<tr>
<td>BRD2/E2</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
</tr>
<tr>
<td>BRD2/E3</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
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<tr>
<td>BRD2/E4</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
</tr>
<tr>
<td>BRD2/E5</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
</tr>
<tr>
<td>BRD2/E6</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
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<tr>
<td>BRD2/E7</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
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<tr>
<td>BRD2/E8</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
</tr>
<tr>
<td>BRD2/E9</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
</tr>
<tr>
<td>BRD2/E10</td>
<td>5’CTTACAGGCTGCTAACTG</td>
<td>219 bp</td>
<td>28 and 62.4</td>
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</table>

The PCR reaction mixture (total volume, 10 μl) containing 50 ng genomic DNA, 2.5 μl of 10X PCR reaction buffer, 1.3 mM MgCl2, 1.0 μl of 25 mM of deoxyribonucleotide triphosphate (dNTP), primers forward and reverse at 5 pmol/μl, digestion of the PCR product was done with 0.4 μl of Taq enzyme (TAKARA, India) in the reaction buffer and 7.9 μl of sterile water for dilution. All the samples were amplified in applying following conditions: 7 min denaturation step at 35 cycles, each cycle: denaturation 95°C for 30 sec, annealing 55-62°C for 45 sec, elongation 72°C – 1.3 min, repeated for 35 cycles followed by a final extension step at 72°C for 7 min. (primer sequences were obtained from this study carried out by Gillis et al 2004). The amplified PCR products were analyzed on 2% agarose gels electrophoresis, stained with ethidium bromide and visualized with UV light. Sequencing was performed in the Sandor Proteomics Laboratory, Banjarahills, Hyderabad (T.S) due to non availability of molecular genetics laboratory in the Department of Anatomy, Krishna Institute of Medical Sciences, Karad. M.H.

Fig:1 Gel picture showing the amplification produces of 12 exons of 3 JME patients

Epidemiology

JME patients begin the seizures between ages 12 and 18 with a mean age at onset 14.6 years, with range from 9 to 40 years. The mean age of onset for GTCS is 15.5 years, absence seizures 11.5 years, and myoclonic seizures 15.4 years.

RESULT

DNA sequencing analysis of BRD2 gene

The complete coding sequence of BRD2 gene is amplified from genomic DNA using standard protocols. We randomly selected three samples in out of 75 JME patients and the entire 12 exons were sequenced initially. Based on the result of molecular analysis in three samples, we found nonsense mutations in exon 9 and decided to process the remaining 72 JME samples and the 100 normal control samples for sequencing to the exon 9 only for identify the mutations.

The sequencing analysis of BRD2 genes was screened by double strand conformational analysis (DSCA) by using ABI 377 automated sequences instrument. Sequenced according to the ABI Big Dye Terminator Cycle sequencing protocol. JME dominated among third degree relatives. BIAST search was used to identify homology between the sequence obtained from the case test are evaluate sequence of case control.

Mutation analysis

We selected initially three JME patients and five unaffected (free from JME) control subjects of the same gender for direct sequencing of all 12 exons in the BRD2 gene. The results revealed a heterozygous nonsense mutations observed only in exon 9. However these mutations...
which is located at 6p21.3 might be susceptibility alleles (odds ratio
In 2003, Pal et al. showed that SNPs within the subcortical white matter of brains
distributed dystopic neurons in gray matter stratum molecular and
developmental transcription regulator expressed in brain and may be
anatomical context of the gene concern. BRD2 is a putative
cerebellum, medulla, occipital cortex, frontal cortex, putamen, brain
cortex, thalamus, red nucleus of midbrain and spinal cord. It has been
abnormalities connected to the motor and pre-motor areas of cerebral
functions.

DISCUSSION
During sleep, 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 (13). Therefore, synchronized activity of large numbers of neurons abolishes their normal 'wakeful' functions.

In recent report of neuron-anatomy reveals that cerebral structure abnormalities, abnormalities connected to the motor and pre-motor areas of cerebral cortex, thalamus, red nucleus of midbrain and spinal cord. It has been reported that BRD2 is plays important role in the development of central nervous system (14).

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 microdyssgenesis (5). 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 (15).

In 2003, Pal et al. showed that SNPs within the BRD2 (RING3) gene which is located at 6p21.3 might be susceptibility alleles (odds ratio 6.5) for autosomal recessive JME in families from New York (5). Linkage disequilibrium found two strongly JME-associated SNP variants in the promoter region of BRD2 and a common variant haplotype in over 50% of 200 probands from families that had produced positive LOD scores for 6p21 during linkage analyses (4). Fourteen patients (18.6%) had a positive family history of epilepsy. The 61 JME (81.3%) patients met the criteria for classic JME.

JME in CrX36 (connexin36) (16). BRD2 deficient embryonic fibroblast cells more observed to proliferate more slowly than controls (Shang et al., 2009) and observed enhanced levels of cell death in brd2 deficient embryos. BRD2 is essential for chromatin structures and transcription during mammalian embryogenesis and neurogenesis. Over expression of BRD2 gene leads to neuronal degeneration and as gene predicts positively regulate the apoptosis of neurons. Neuronal programmed cell death during embryogenesis drives JME. In human the CN5 is 70% to 80% at various stages for the morphogenetic events. 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 (16,17). 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. The effect of BRD2 SNPs on promoter function is currently unknown, but they may help in the altering, tissue structure or level of brd2 expression (7). BRD2 gene between alternative exon 2 and exon 3 were shows highly polymorphic microsatellite and certain alleles were strongly associated with JME (Greenberg et al., 2000).

CONCLUSION
In conclusion, we identified six nonsense mutations in BRD2 in 4 males and 2 females and molecular genetic analysis of BRD2 is important for a definitive diagnosis of JME. The relationship between the genotype and clinical phenotype is not known. Further large scale of Familial and twin studies are required to investigate for strong involvement of BRD2 and BRD3 genes in the genetic susceptibility of JME syndrome. However, the exact impact of the mutation needs to be confirmed by future studies on function.

Conflict of interest statement
No conflict of interest to disclose.

Acknowledgements
We thank all of the JME patients and case subjects for participating in this study. We sincerely thank to Dr. (Mrs). Dosi M.A.; Professor and Head, KIMS, Karad (M.H); We thankful to Dr. (Mrs). Parveen Jahan, Associate Professor, Dept. of Zoology, School of sciences, Maulana Azad National Urdu University, Hyderabad (TS) for the valuable discussion and guidelines. We thank to Dr. B.N. Umarji Professor and Dr. Chenana.C Director, BRIMS, Bidar (K.A).

REFERENCE


15. Meencke HJ, Vert H. The relevance of slight migrational disturbances (microdysgenesis) to the etiology of the epilepsies. Surgical Neuropathology Text book and Atlas; Malformation of cortical development, page: 51
