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Original Article

Mutational Analysis of Gene BRD2 in Patients of Juvenile Myoclonic Epilepsy

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ABSTRACT

Background: Epilepsy is characterized as third most common chronic brain disorder. It is known as by an enduring tendency to reduce seizures. Juvenile myoclonic epilepsy is the most frequent generalized epilepsy syndrome in adolescents or childhood. Juvenile myoclonic epilepsy based on finding of linkage analysis, is a genetic association study of photo paroxysmal response was carried out focusing on the *BRD2* gene and its polymorphism in German population and other world population.

Materials &Method: For this purpose, five epileptic families were identified, Blood samples were collected with two or more epileptic persons from different areas of Balochistan, and DNA was isolated by Inorganic method. Total 5 exons of *BRD2* gene, harboring extensively reported mutations, were selected for genotypic analysis. PCR amplification and DNA sequencing of gene *BRD2* (Bromodomain Containing Protein 2) was carried out to confirm any genetic variability in patients with familial inheritance in selected families.

Results: During this study four different mutations were identified (c.610+35 insertion, c.145G>T, 146C>G, p.Ala49Cys, p.lys31lys, c.1062 T>C, p.Asn35Asn) in families with myoclonic epilepsy. Two of the variants are silent mutations; one is 1bp insertion in intron-4 while a missense substitution was also identified. The mutation is probably damaging which is confirmed by an online protein prediction tool Polyphen-2 prediction.

Conclusions: It is concluded that highest percentage of Juvenile myoclonic epilepsies (JME) is in *consanguineous* marriage families. An important factor contributing to high incidence of Juvenile myoclonic epilepsies (JME) is the unawareness among people about the available diagnostic facilities for the prenatal diagnosis in Pakistan. Further studies involving larger number of patients aiming at genetic categorization of these patients may be helpful in better understanding of underlying pathophysiology.

Key Words: *BRD2* gene, Epilepsy, Electroencephalogram (EEG), Juvenile myoclonic epilepsy (JME), Nervous system, Mutation.

Introduction

Epilepsy is a brain disorder characterized by a persistent predisposition to create seizures, and by emotional and cognitive dysfunction[1]. In 2005, the International League Against Epilepsy (ILAE) defined epilepsy as a brain illness characterized by a persistent predisposition to generate epileptic seizures. This has social, psychological, and neurological consequences.[2]

Clinical manifestation of seizure starts when abnormality occurs in cortical neuronal activity; the neuron becomes hyperactive and spreads all over the brain. "However'' first manifestation begins at single neuron level; brain is systemized in a sequence of interrelated network of neurons. When abnormality occurs, then single neuron becomes hyperactive and it spreads into all interconnected neurons, after it spread into majority of neurons it then causes clinically visible seizures [3]. Epileptic seizures have been classified on several different dimensions (Partial and Generalized seizures).[4]

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Interactive appearances of a seizure are strong-minded by the functions normally work for the cortex at which the seizure begins. For instance, a seizure rising in the motor cortex is obvious by way of jerking of the body part which is controlled by cortical region of beginning. Partial seizure is categorized into simple and complex seizure. Simple partial seizure is related with preservation of consciousness while partial seizure is related to loss of consciousness. Most of complex partial seizures initiates in the temporal lobes. Tonic-clonic seizure is example of generalized seizures. Myoclonic seizures are another type of generalized seizures which are characterized by brief, sudden, shock like muscle contraction in patients. Myoclonic seizures either single or repeated jerks affecting one side or more are widespread and affecting both sides. In tonic seizures sudden uncontrolled muscle contraction consisted while in clonic seizures the absence of tonic and only clonic jerks can occur with repetition. Tonic-clonic is more common seizure of generalized type usually comprises unconsciousness and all body muscle groups[4].

In epilepsies all apparent modes of inheritance are found, containing Autosomal, x-chromosomal, mitochondrial and complex inheritance. Most common mode of inheritance for epilepsies among these is autosomal recessive inheritance which can be passed by healthy carriers through generations and in homozygous states the early disablement or death can cause by early age onset and progressive course. There are some syndromes in which epilepsy is more commonly associated with neurological signs including mental retardation, dementia or ataxia and epileptic seizures are also important in such more than 200 inherited syndromes. Genes that can cause such syndromes are also involved in performing some others functions in body such as Glycogen metabolism, Brain development and respiratory chain activity. JME (Juvenile Myoclonic Epilepsy) is one example of such syndrome disorders.

Juvenile myoclonic epilepsy in adolescents or childhood is the most recurrent generalized epilepsy syndrome with an estimated prevalence of 1-per 1000,000, accounting for 5-10 % of all epilepsies[5]. Its predominant feature is myoclonic jerks of the proximal upper extremities, mainly occurring in the morning hours. Most patients have occasional generalized tonic-clonic seizures and about one third of them report of absence seizures[6].

By medication are controlled all seizures in two of three patients and by medication 80% are free of generalized tonic-clonic seizures while occasional myoclonic jerks persist. Some patients show pseudo-resistance due to problems with treatment, compliance or lifestyle.[7] but only one in six is truly refractory to medication[8.] The presence of all three different seizure types has repeatedly been associated with an unfavorable response to medication. [9, 10, 8]

The pathophysiology shows that JME is multifactorial and includes both Mendelian and complex genetic inheritance factors, even though the currently known genetic alterations can only be shown in a minority of patients[11]. In *BRD2* gene, at N-terminal all of which contain tandom bromo-domains and at C-terminal an extra terminal domain of unknown function.[12] *BRD2* has important cellular functions as showed in the null mice which are homozygous embryonic lethal.[13, 14]

Materials and Methods

Identification and enrolment of families

Five epileptic families were identified with two or more epileptic persons. Their age ranges from 1 year to 25 years, having symptoms from moderate to severe from different areas of Balochistan. 3 mL blood was drawn intravenously into 50ml tube containing 400µL EDTA.

Pedigree analysis

The Pedigrees were drawn using Cyrillic 2.1 software program on the basis of data and information collected from the patients and families.

Clinical evaluation

Medical history from all affected individuals of the enrolled families was obtained that cause epilepsy and also clinical test by electroencephalogram (EEG) and computed tomography (CT) scan was done to confirm epilepsy.

Laboratory work:

In the laboratory, the standard two days Inorganic DNA extraction protocol was used to extract genomic DNA from samples for PCR amplification.[15]

Primer designing, DNA isolation and exons amplification:

Suitable primers were designed using Primer 3 and Primer 3 plus computer program
(http://.bioinfo.ut.ee/primer3/)
(http://.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi/).

As there was one gene of our interest (*BRD2*), containing 7 exons. Total of 7 exons oligonucleotide primers were designed as shown below in table.

Table1: Sequences of primers used for the amplification of *BRD2*gene.

Exons	left primer (5-3)	Right primer (5-3)	Size
Exon-2	CAGCACCTGCATTCATCAGA	CTGCCCTACAGGGGAGAAAT	620 bp
Exon-3	GGGGCCGCAGTTTAAGTAAC	TGAAGTCACATCACTACTGTGTTCAA	514 bp
Exons-4,5	CTGGTGGGGGTATGGTAATG	CCAGAGGAAATCCACAGATCA	571 bp
Exon-6	GCCAAGGTATGATCTGTGGAT	AACACCAGACCTTCTGTTCCA	627 bp
Exons-7,8	ATCCCCACTGTGCTCTCAA	AATAAAAACTTTCAAGAGTGACAAAA	700 bp

Genomic DNA was amplified through polymerase chain reaction (PCR) using exon2, exon3, exon 4-5, exon 6 and exon7-8 primers. PCR reactions were performed with 50 ng of template DNA in 20 µL reaction mixture for 10 samples containing 10 µM Primers, 4µL PCR buffer (100mM Tris-Cl, pH 8.4, 500mM KCL, 20nM MgCL2 and 1% Triton), and 1.6µL of 2mM dNTPs and 5 units of Taq DNA Polymerase. Amplifications of exons were performed with an initial activation step at 93°C for 3 min followed by first 10 cycles as touchdown PCR (with annealing temperature from 64°C to 54°C or 67°C to 57°C) and additional 20 cycles with denaturation at 95°C for 30 sec, annealing at 54°C for 30 sec and extension at 72°C for 45 sec with a final extension at 72°C for 7 minutes. Amplified products were run on 2% agarose gel in TBE buffer at 110 V for 45 minutes and were visualized by staining with ethidium bromide under UV trans-illuminator.

DNA Sequencing:

For DNA sequencing, the *BRD2* gene from genomic DNA was amplified via PCR using exon specific primers, then such PCR product was used as a template DNA for sequencing[16, 17]. Sequencing reaction were prepared by using 15µL from the above prepared EXO-SAP solution the following reagents were added in specific amounts as, 6µL of diluted PCR product, 2µL of big dye sequencing mix, 1µL of primer (3.2µM), and 1µL of 5X dilution buffer. PCR were carried using thermo-cycler program of 30 basic cycle of standard PCR. After exons amplification, all exons and exon/ intron border regions were sequenced and analyzed by BioEdit (version 7.0.2) for mutation analysis.

Results

Five autosomal recessive epileptic families from different areas of Balochistan (Three families from Pishin, one family from Quetta and one family from Loralai) were enrolled in this study. Epileptic patients were first confirmed clinically by examining via conducting EEG tests.

In this study we identified four sequence variants/mutations in affected and normal individuals in families with juvenile myoclonic epilepsy (JME). In family-B a 1bp insertion at c.610+35 was identified in intron 4 of *BRD2* gene. In family-D a missense substitution was identified, replacing amino acid alanine by cysteine at position 49(p.Ala49Cys), while in same family a silent mutation was also identified (p.lys31lys). It is to further explain that in the same family another silent mutation was identified in exon 6 of gene *BRD2* (p.Asp35Asp, c.1062T>C). Such mutation is probably damaging and their pathogenicity of *BRD2* was confirmed by an online protein prediction tool polyphen-2 prediction (http://genetics.bwh.harvard.edu/pph2) "which predicts possible impact of an amino acid substitution on the structure and function of a human protein" showed that such mutation is probably damaging to the protein's functions.

Table 2: Showing the results of mutational analysis of five exons of *BRD2* gene.

Pedigree Names	Mutational Analysis of Gene *BRD2*				
	Exon 2	**Exon 3**	**Exon 4-5**	**Exon 6**	**Exon 7-8**
Family A	Wild type	Wild type	Wild type	Wild type	Wild type
Family B	Wild type	Wild type	1bp insertion at c.610+35	Wild type	Wild type
Family C	Wild type	Wild type	Wild type	Wild type	Wild type
Family D	c.145 G>T, 146 C>G, p. Ala49Cys (P. Lys 31 Lys)	Wild type	Wild type	c.1062T>C and (P. Asp 35 Asp)	Wild type
Family E	Wild type	Wild type	Wild type	Wild type	Wild type

Family–B was collected from Pishin. Blood samples of six individuals were collected. Out of these two were affected and other four were normal. The sequencing results that shows having 1bp insertion at c.610+35 in intron 4 in affected and normal individual which also resulting in wild type *BRD2*.

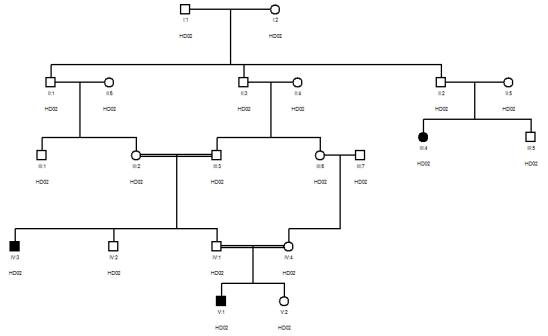

Figure 1: Pedigree of Family-B, with three affected and 18 normal individuals.

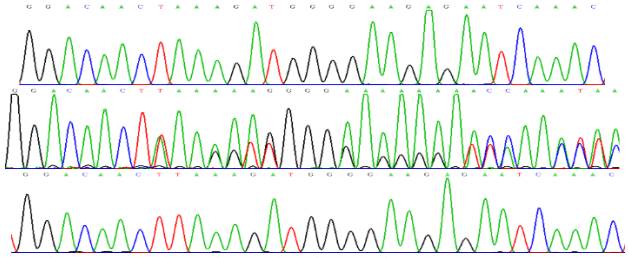

Figure 2: Showing 1bp insertion at c. 610+35 in affected individual of family-B.

Family –D was collected from Pishin. Blood samples of five individuals were collected. Out of these two were affected and the other three were normal. Sequencing results of this family were analyzed by BioEdit software that shows having a synonymous missense substitution mutation in Exon 2 where Guanine is replaced by Thymine and Cytosine is replaced by Guanine, consecutively i.e. c.145G>T andc.146C>G resulting p.Ala49Cys and silent mutation (P. lys 31 lys) in exon 2 and also silent mutation in exon 6 that is c.1062T>C (p. Asp 35Asp) which also resulting in wild type *BRD2*.

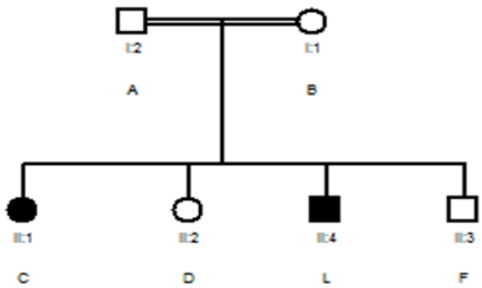

Figure 3: Pedigree of family-D, containing two affected and four normal individuals.

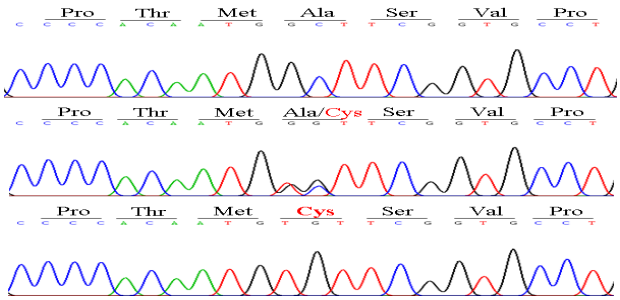

Figure 4: Showing missense mutation in exon 2 at c.145 G>T, 146 C>G, p. Ala49Cys

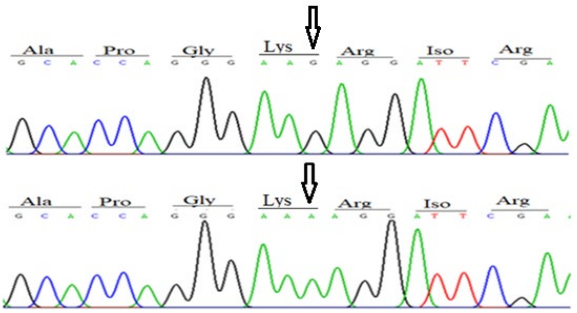

Figure 5: Electropherograms for silent mutation (P. lys 31 lys) in affected individual in exon 2.

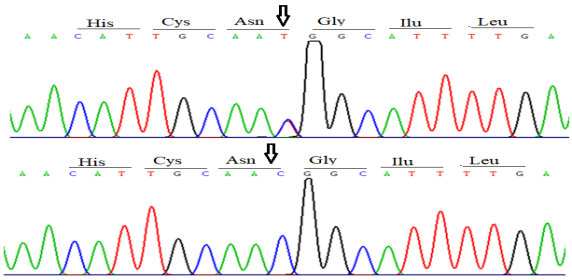

Figure 6: Showing silent mutation in exon 6 at c. 1062 T>C and (p. Asp 35Asp).

But in Family –A, Family –C and last family, after sequencing no mutations were detected in *BRD2*gene.

Discussion

Juvenile myoclonic epilepsy (JME) is well defined age-related idiopathic generalized epilepsy syndrome in adolescents or childhood.[18] Myoclonic jerks occurring shortly after awakening, frequently involving in the upper extremities. The most common type of seizure in people with this condition have generalized tonic-clonic seizures and about one third of them report absence seizures [19].

Initially the correct diagnosis of JME is mostly missed, with a mean delay of 8 years from first symptoms to

correct diagnosis[20].Typically age of onset is between 12 to 18 years, although late onset up to the fourth decade of life has been described[21].There is a subgroup of JME patients, evolving from previous childhood absence epilepsy (18% in)[22]. Which may lead to confusion regarding the age of onset.

Juvenile myoclonic epilepsy based on the finding of linkage analysis, is a genetic association study of photo paroxysmal response was carried out focusing on the BRD2 gene and its polymorphism in a German population[23]. Based on the comparison of 666 healthy control cases, the initial results indicated that both JME and PPR (including Doose type 1-4) are could be an underlying susceptible *BRD2* gene.

BRD2 gene located on chromosome 6p21.32, Sequencing reaction was performed to determine if any mutation was found in any enrolled family. During this study four different mutations were identified (c.610+35 insertion, c.145G>T, 146C>G, p.Ala49Cys, p.lys31lys, c.1062 T>C, p.Asp35Asp) in families with myoclonic epilepsy. Two of the variants are silent mutations, one is 1bp insertion in intron-4 while a missense substitution was also identified. The mutation is probably damaging which is confirmed by an online protein prediction tool Polyphen-2 prediction.

In family-B we identified an insertion mutation in intron 4 in affected and normal individual and in Family-D we identified a missense substitution mutation where guanine is replaced by thymine and cytosine is replaced by guanine, consecutively, i.e. c.145G>T, 146C>G in exon 2, leading to p.Ala 49 Cys and silent mutation in exon 2 and also identified silent mutation in exon 6 of *BRD2* gene i.e. .c.1062T>C in affected and normal individual. This mutation is probably damaging and their pathogenicity of *BRD2* was confirmed by an online protein prediction tool polyphen-2 prediction (http://genetics.bwh.harvard.edu/pph2) "which predicts possible impact of an amino acid substitution on the structure and function of a human protein" showed that such mutation is probably damaging to the protein's functions.

Another study Roshan *et al.,* (2017) found three novel missense mutations of *BRD2* gene in 2.2% of unrelated Juvenile myoclonic epilepsies (JME) patients from south India. The present study is the first report in relation to JME and *BRD2* gene from this part of the world. Large scale family studies are required to establish the present observation in different ethnic populations. The genetic background for Juvenile myoclonic epilepsies (JME) accounts for 5-10% of all form of epilepsy. A non-ion channel gene of the *BRD2* protein cause programmed cell death in the developing brain. JME inheritance is autosomal dominant in all *BRD2* mutations show heterozygosity in affected individuals. In the current study direct sequencing of the *BRD2* gene exhibited a heterozygous missense mutations (C>A, A>T, G>A) in exon 11-12 that may alter the amino acid there by the function of the protein molecule.

Five novel mutations in transcripts A and B of Myoclonin1/EFHC1 were found in another study Medina *et al.,* (2008). In transcript A two novel heterozygous missense mutations (c.1523C>G and c.755C>A) both a singleton from Mexico the other one from Japan. In a mother and daughter from Mexico in transcript B, a deletion/frameshift (C.7898del.AV264fsx280) was present. In transcript B of a large Honduran family, in seven affected epileptiform-EEG and in four clinically segregated members a nonsense mutation (c.829C>T) were found. In a sporadic case the same non mutation (c.829C>T) occurred as a de novo mutation. In a family from Japan, finally we found a three-base deletion (-364%-362del.GAT) in the promoter region.

Greenberg *et al.,* (2000) conducted study on 55 patients from different ethnic groups residing in, (Foster City, CA, and U.S.A.) were available for all three SNPs (assay ID C1024346 20,C102433810, C102434620), in this study was around 55% if genotype relative risks were2.0 in a dominant model. If a mutation in *BRD2* or its surrounding genes specifically causes epilepsy.

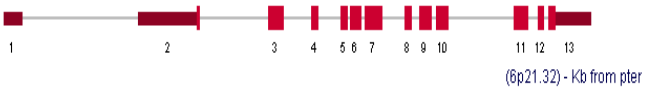

Figure7: Showing the different domains of *BRD2* gene

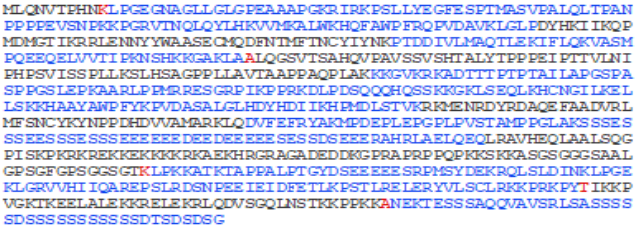

Figure 8: Showing the sequencing of amino acids of *BRD2* gene

Recommendations

During this study four different mutations were identified in families with myoclonic epilepsy. Two of the variants are silent mutations, one is 1bp insertion in intron-4 while a missense substitution was also identified in the *BRD2* gene, however, most of our families with JME did not carry any *BRD2* gene mutations but further studies of the *BRD2* gene to find out new mutations are important. The results of our study can be extend the distribution of *BRD2* gene mutations to the Pakistani population and confirm the high level of genetic heterogeneity associated with Juvenile myoclonic epilepsies.

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Conflict of interest:
The authors declare that they have no potential conflicts of interest.

References

1. Vezzani A, Aronica E, Mazarati A, Pittman QJ. Epilepsy and brain inflammation. Exp Neurol. 2013; 244:11-21.
2. Hesdorffer, DC, Benn, EKT, Cascino, GD. et al. Is a first acute symptomatic seizure epilepsy? Mortality and risk for recurrent seizure. Epilepsia. 2009; 50: 1102-1108.
3. Steinlein OK. Genetic mechanisms that underlie epilepsy. Nat. Rev. Neurosci. 2004; 5(5): 400-408.
4. JO McNamara. Cellular and molecular basis of epilepsy Journal of Neuroscience. 1994; 14 (6): 3413-3425.
5. Jallon P and Latour P. Epidemiology of idiopathic generalized epilepsies. Epilepsia. 2005; 46(9): 10–14.
6. Genton P, Thomas P, Kasteleijn-NolstTrenite DG. et al. Clinical aspects of juvenile myoclonic epilepsy. Epilepsy Behav. 2013; 28(1): 8–14.
7. Baykan B, Martinez-Juarez I.E. et al. Lifetime prognosis of juvenile myoclonic epilepsy. Epilepsy Behav. 2013; 28(1): 18–24.
8. Gelisse P, Genton P, Thomas P. Clinical factors of drug resistance in juvenile myoclonic epilepsy. J. Neurol. Neurosurg. Psychiatry. 2001;70:240–3
9. Baykan B, Altindag EA, Bebek N, Ozturk AY, Aslantas B. et al. Myoclonic seizures subside in the fourth decade in juvenile myoclonic epilepsy. Neurology. 2008; 70: 2123–2129.
10. Camfield CS, and Camfield, PR. Juvenile myoclonic epilepsy 25 years after seizure onset: a population-based study. Neurology. 2009; 73: 1041–1045.
11. Delgado-Escueta AV, Koeleman BPC.et al. The quest for juvenile myoclonic epilepsy genes. Epilepsy Behav. 2013; 28(1): 52–57.
12. Shang E, Wang X, Wen D. Double bromodomain-containing gene Brd2 is essential for embryonic development in mouse. Developmental dynamics: an official publication of the American Association of Anatomists. 2009; 238(4): 908–917.
13. Gyuris A, Donovan DJ, Seymour KA, Lovasco LA, Smilowitz NR. et al. the chromatin-targeting protein Brd2 is required for neural tube closure and embryogenesis. Biochim Biophys Acta. 2009; 1789 (5): 413- 421.
14. Shang E, Wang X, Wen D, Greenberg DA. et al. Double bromodomain-containing gene Brd2 is essential for embryonic development in mouse. DevDyn. 2009; 238(4): 908-17.
15. Grimberg J, Nawoschik S, Belluscio L. et al. Simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acids Res. 1989; 17:83-90.
16. Cannelli N, Cassandrini D, Bertini,E. et al. Novel mutations in CLN8 in Italian variant late infantile neuronal ceroid lipofuscinosis: another genetic hit in the Mediterranean. Neurogenetics. 2006; 7(2):111-117.
17. Reinhardt K, Grapp M, Schlachter K, Bruck W. Novel CLN8 mutations confirm the clinical and ethnic diversity of late infantile neuronal ceroid lipofuscinosis. Clinical genetics. 2010; 77 (1): 79-85.
18. Janz D. Epilepsy with impulsive petit mal (juvenile myoclonic epilepsy). Acta Neurol. Scand. 1985. 72:449–59.
19. Genton P, Thomas P, Kasteleijn-NolstTrenite DG. et al. Clinical aspects of juvenile myoclonic epilepsy. Epilepsy Behav. 2013; 28 (1): S8–14.
20. Panayiotopoulos CP, Tahan R, Obeid, T. Juvenile myoclonic epilepsy: factors of error involved in the diagnosis and treatment. Epilepsia. 1991; 32: 672–6.
21. Delgado-Escueta AV, Enrile-BacsalF. Juvenile myoclonic epilepsy of Janz. Neurology.1984; 34: 285–94.
22. Martinez-Juarez IE, Alonso ME, Medina MT, Duron RM. at al. Juvenile myoclonic epilepsy subsyndromes: family studies and long-term follow- up. Brain. 2006; 129: 1269–80.
23. Lorenz S, Taylor KP, Gehrmann A, Becker T, Muhle H. Association of BRD2 polymorphisms with photoparoxysmal response. Neuroscience Letters. 2006b; 400:135-139.
24. Roshan ZM, Dosi MA, Jahan P. et al. Novel missense mutations in BRD2 gene of JME patients: A study from South India. International Journal of Medical and Health Research. 2017; 3 (5): 89-94.
25. Medina MT, Suzuki T, Alonso ME, Duron RM. et al. Novel mutations in Myoclonin1/EFHC1 in sporadic and familial juvenile myoclonic epilepsy. Neurology. 2008; 7022:2137-44

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B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion