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Early-onset epileptic encephalopathy related to germline *PIGA* mutations: a series of 5 cases

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1. Abstract

The molecular diagnosis of early-onset epileptic encephalopathy (EOEE), an expanding field in child neurology, is becoming increasingly possible thanks to the widespread availability of next-generation sequencing and whole exome sequencing. In the past 15 years, mutations in *STXBP1*, *KCNQ2*, *SCN2A*, *SCN8A* and numerous other genes have been reported, giving a more accurate insight for these rare diseases. Among these genes, germline mutations in *Phosphatidylinositol Glycan A (PIGA)* gene were first reported in 2012. Located on Xp22.2, *PIGA* is involved in the synthesis of GPI (glycosylphosphatidylinositol) which acts as a membrane anchor for different proteins: enzymes, adhesion molecules, regulation of the complement way, and co-receptor in transduction signal. Boys suffering from this condition exhibit developmental delay with early-onset epilepsy, severe dysmorphic signs, multi-visceral anomalies and early death in the most severe forms. We report five cases of germline *PIGA* mutations, with four missense mutations not reported to date. We discuss the clinical and paraclinical features including MRI and electroencephalographic data. The differential diagnosis can be challenging, notably when dysmorphic signs are subtle, and we show that *PIGA* deficiency may mimic metabolic disorder, mitochondrial disease, and focal or migrating epilepsy. Interictal EEG may be normal at onset of epilepsy. Widespread use of whole exome sequencing would avoid the risk of an inaccurate diagnosis. The phenotype is heterogeneous, and the distinction between “severe” and “less severe” forms needs to be assessed, as patients may exhibit intractable epilepsy with severe developmental delay without multi-organ involvement or marked dysmorphic signs.

2. Keywords

PIGA- Glycosylphosphatidylinositol-Encephalopathy –Early-onset epilepsy- Whole exome sequencing-Next-generation sequencing ¹

¹ Abbreviations : CADD : combined annotation dependent depletion EEG : electroencephalography EIMFS : epilepsy of infancy with migrating focal seizures EOEE : early-onset epileptic encephalopathy ExAC : exome aggregation consortium MRI : magnetic resonance imaging NGS : next-generation sequencing PET-scan : positron-emission tomography PolyPhen2 : polymorphism phenotyping v.2 SIFT : sorting intolerant from tolerant SUDEP : sudden unexpected death in epilepsy WES : whole exome sequencing

3. Introduction

Early-onset epileptic encephalopathy (EOEE) is a serious condition that may severely disable children with developmental delay and severe epilepsy. The diagnosis is challenging, but the increasing use of highly-efficient genetic analysis thanks to next-generation sequencing or whole exome sequencing allows early precise genotypic to be made more frequently. *PIGA* (*Phosphatidyl inositol Glycan class A*) gene is a 162-Kb gene located on Xp22.2. *PIGA* is involved in the synthesis of glycosylphosphatidyl inositol, an anchorage protein in the cell's membranes. *PIGA* gene encodes the catalytic subunit of the GPI-GlcNAc transferase (GPI-GnT) complex for the synthesis of the first GPI precursor, GlcNAc-PI (N-acetylglucosaminyl phosphatidylinositol). GPI is involved in numerous molecular pathways, such as signal transduction, regulation of the complement, and neurogenesis. Somatic mutations in *PIGA* lead to a rare disease, paroxysmal nocturnal hemoglobinuria (MIM 300818). Germline mutations in *PIGA* (MIM 311770) were formerly considered as lethal during embryonic life, but the first report by Johnston *et al.* laid the foundation for a new condition primarily called early-onset epileptic encephalopathy with severe muscular hypotonia, multiple congenital anomalies, and early death (MCAHS2, MIM 300868) [1]. However, the phenotype seems heterogeneous and different terms were coined thereafter: neurodegenerative encephalopathy with systemic iron overload (ferro-cerebro-cutaneous syndrome) [2] ; intellectual disability and seizures without dysmorphism [3] ; and Simpson-Golabi-Behmel Syndrome type 2 [4]. We report five new cases of germline *PIGA* mutations, with a particular focus on clinical aspects including differential diagnosis, in order to identify common features and clarify genotype-phenotype correlations and prognosis.

4. Material and Methods

Five patients exhibited germline *PIGA* gene mutations. All files were retrospectively analyzed, including genotypic analysis, developmental aspects, epilepsy, electroencephalographic and MRI evaluations, treatments and evolution.

For patient #1, 151 genes were analyzed with the kit Sure Select Epileptome-V1.hg19-ID0757311 551.106 Kb (Agilent technologies, Santa Clara, CA, USA) and sequencing with HiSeq (Illumina), in Paris, France (Necker Hospital for Sick Children, Molecular Genetics Unit, Pr Jean-Paul Bonnefont and Dr Giulia Barcia).

Samples for patients #2, 3 and 5 were analyzed by Dr Laurent Villard in Marseille; next-generation sequencing was performed using a SureSelectXT Custom 12-24Mb library (Agilent Technologies) on a ion proton platform (Thermo Fisher). The captured exons correspond to a list of 116 genes involved in epileptic encephalopathies (list available upon request). The results were analyzed using the Torrent suite (Thermo Fisher) for alignment and variant calling. To filter and establish coverage and variant analysis, we used the Varaft annotation and filtration system (Desvignes et al., *Nucleic Acids Res.* 2018 Jul 2; 46: W545–W553. doi: 10.1093/nar/gky471)

Concerning patient #4, library generation, exome enrichment and whole exome sequencing were performed at the French National Centre for Genotyping (CNG, Evry, France). Libraries were prepared from 3 µg genomic DNA extracted from whole blood using an optimized SureSelect Human Exome kit (Agilent) following the manufacturer's instructions. Captured, purified and clonally amplified libraries targeting the exome were then sequenced on a HiSeq 2000 (Illumina) according to the manufacturer's recommendations. Obtained sequence reads were aligned with the human genome (hg19) using BWA software. Downstream processing was carried out with the genome analysis toolkit (GATK), SAMtools and Picard Tools (<http://picard.sourceforge.net/>). Single-nucleotide variants and indels were subsequently called by the SAMtools suite (mpileup, bcftools, vcftutil). All calls with a read coverage $\leq 5x$ and a Phred-scaled SNP quality of ≤ 20 were filtered out. Substitution and variation calls were made with the SAMtools pipeline (mpileup). Variants were annotated with an in-house Paris Descartes bioinformatics platform pipeline based on the Ensembl database (release 67).

5. Results

5.1 Overview of the five patients

Clinical and paraclinical findings are reported in table 1. All patients were boys, born after non consanguineous unions, from five French unrelated families. Patient #4 had increased birth parameters. Mild dysmorphic signs were present in all cases. Increased rates of alkaline phosphatases were transiently present in two cases. Patients #1 and #2 exhibited renal cysts. Developmental delay with intractable seizures appearing between 4 months and one year of age was present in every case. Patients #1 and #3 died at age 8 and 14 years respectively, while patient #2 and #4 are not ambulatory and cannot speak, with severe epilepsy (age 5 and 8 years at last evaluation). Patient #5 was aged 3 years at last evaluation and exhibited developmental delay but a controlled epilepsy.

5.2 Dysmorphic signs and organ malformations

Photos of four patients are shown in figure 1. Photos of patient #5 were not available. A tented upper lip was present in every patient and a prominent forehead in 4 out of 5 children. An anteverted nose was noted in 2 out of 5 patients. Other shared features were dysplastic ears (2/5) and a small chin (2/5). A non-specific posterior plagiocephaly was also present during follow-up in all patients except patient #5 (who is the youngest in the series); it was linked to axial hypotonia. No cardiac or digestive malformations were present. Renal cysts were present in two patients and patient #1 suffered from renal lithiasis.

5.3 Epilepsy and electroencephalographic studies

Figure 2 shows the heterogeneity of EEG in the series. Epilepsy was of early-onset in all the patients, with first seizures occurring between four months and one year. However, no neonatal epilepsy was present. Onset of epilepsy was heterogeneous: either spasms, considered “atypical” for patient #2, and in extension for patient #5; or motor seizures with apnea or oculoclonus. Myoclonic jerks were rare, and noted only in patient #2. An EEG was performed in patient #2 during first investigations for developmental delay, hence before onset of epilepsy. It showed short bursts of spike-waves of high amplitude, while the child was awake and during sleep, but spindles were present. Patient #3 exhibited “spasms” between one and three months, not considered as epileptic and EEG at age 4 months revealed a poor spatial organization with paroxystic abnormalities in the left hemisphere. Patient #1 exhibited paroxystic and multi-directional nystagmus at age 4 months, but electroencephalographic study was then normal. Nystagmus lasted for a few weeks and self-terminated. The diagnosis of epilepsy in infancy with migrating focal seizures (EIMFS) was considered at age 10 months: he exhibited eye and head deviation to his right side, and then to his left side alternatively, sometimes associated with a deep inspiration.

During follow-up, three main types of seizures were present in our series: atypical spasms, unilateral upper limb clonus, and oculoclonus associated with apnea and/or impaired awareness.

The diagnosis of focal epilepsy was considered in two patients (#1 and #4), due to abnormal findings on PET-scan, and movements evocative of focal seizures. However, the poor correlation between images, EEG data and clinical findings did not lead to a surgical procedure. Such investigations had been performed before molecular diagnosis.

5.4 Magnetic Resonance Imaging (MRI)

MRI were normal, or showed non-specific abnormalities (figure 3). It was performed in all cases after onset of epilepsy. Patients #4 and #5 exhibited a thin corpus callosum. The latter had also findings evocative of a mitochondrial pathology, including diffusion restriction on periaqueductal zones and pallidum, suggestive of Leigh syndrome. A peak of choline of uncertain origin was present in patient #2. When performed, follow-up evaluations revealed non-specific atrophies (patients #1 and #4).

5.5 Anti-epileptic treatments

A wide range of antiepileptic treatments was tried with different combinations. Vigabatrin was given to all patients except patient #1, and all but patient #5 received valproate. Two patients were treated with a ketogenic diet. It had little effect on seizure frequency but both boys had a better contact thereafter. A vagal nerve stimulator was implemented at age 5 years for patient #1, with a transitory response on epilepsy and contact. Carbamazepine and/or oxcarbazepine were given for all patients except for patient #5, who is the youngest in the series and exhibits less severe epilepsy. Patient #1 received stiripentol in association with carbamazepine because his electroencephalographic pattern was evocative of migrating partial seizures of infancy. More recent treatments, including fenfluramine, cannabidiol, eslicarbazepine, or quinidine, have not been proposed to these patients.

5.6 Genetic analysis

All five patients harbored hemizygous missense mutations. In three cases, the mutation was inherited from the mother (#2 c.145G>A p.Val49Met exon 2, #4 c.356G>A p. Arg119Gln exon 2, #5 c.56G>A p. Arg19Glu exon 2). The two others were *de novo* mutations (#1 c.1178G>T p.Arg393Ile exon 5, #3 c.565A>G p.Lys189Glu exon 2). The mutations in patients #1-2-3- and 5 were absent in dbSNP/Exac but were considered deleterious for protein function using both PolyPhen2 and SIFT tools. CADD (combined annotation dependent depletion) score was higher than 20 in patients #1-4, confirming the highly pathogenic mutations. It was lower in patient #5 (5.791) due to a less conserved region, so the algorithm was less reliable.

6. Discussion

Overall, the patients in this series exhibited rather subtle dysmorphic signs. The only feature shared by all children was a tented upper lip, which has a poor specificity and can be encountered in numerous conditions. The first patients reported by Johnston in 2012 had multi-organ involvement and extremely severe facial dysmorphism [1]. More recent reports have shown that dysmorphic signs may be absent [5,6]. Between these two extremities, a wide spectrum of facial features has been described. Most of the findings demonstrated by our patients have already been reported, including a prominent forehead (sometimes referred to as “high anterior hairline”), small anteverted nose, dysplastic low-set ears with thick ear lobes, small chin (or “micrognathia”), long philtrum, deep palmar and plantar creases. Other frequent dysmorphic signs not encountered are depressed nasal bridge, high arched palate or Pierre Robin sequence, and malar flattening.

Epilepsy is the most common feature in *PIGA* deficiency. No case of *PIGA* deficiency without seizures has been reported to date. In all our cases, epilepsy was heralded by developmental delay. Patient #1 exhibited multi-directional nystagmus (at age 4 months) with hypotonia and the diagnosis of epilepsy was suspected, but first electroencephalographic proved normal. Like in *CDKL5* mutations, normal interictal EEG can be present during the first stages of epilepsy in *PIGA* deficiency [7]. None of the patients had myoclonic epilepsy even if patient #2 could exhibit sporadic myoclonic jerks. During follow-up, electroencephalographic studies demonstrate a wide range of patterns that are modified by brain maturation, medications, and the course of the disease. However, there are three main types of seizures: atypical spasms, oculoclonic seizures with apnea, and upper limb clonus.

The pathophysiology is complex. Chiyonobu hypothesized that abnormal alkaline phosphatases are anchored inside the cellular membrane, inducing an intracellular pyridoxal phosphate deficiency, the latter being required for GABA synthesis [8]. According to Kato *et al.*, the severity of the phenotype is linked to residual *PIGA* protein activity [9]. *In silico* studies were not performed in our series. We did not encounter any neonatal epilepsy with burst suppression. Very early onset of seizures, including neonatal epilepsy, seems to be linked to c. 1234C>T p.Arg412x mutation [1,9]. In the study by Olson *et al.* assessing genetics in early onset epileptic encephalopathy with burst suppression, and a *PIGA* mutation was present in only one out of the 20 resolved cases [10]. Anomalies in *KCNQ2*, *STXBP1* and *SCN2A* are more frequent in such cases. Sudden unexpected death in epilepsy (SUDEP) may be present in early onset epileptic encephalopathies, for instance in *SCN8A* mutations [11]. To our knowledge, no cases of SUDEP have been reported in *PIGA* mutations, probably because it is not a channelopathy. The two patients deceased in our series died after respiratory failure.

MRI findings in *PIGA* mutations represent a rather restricted field. The anomalies that we found in our series have already been reported. For example, Tarailo-Graovac *et al.* reported restricted diffusion on the brainstem tegmentum, superior cerebellar peduncles, subthalamus and ventral striatum, similar to patient #5 [12] and a diminished N-acetyl-aspartate peak on iterative spectrometry in the midbrain, as in patient #1. Other findings like a normal initial MRI, a thin corpus callosum, delayed myelination, and the evolution to a non-specific cerebral atrophy, have also been reported [1, 2, 9].

Differential diagnosis is difficult. Two patients exhibited focal abnormal metabolisms on PET-scan. Such findings must be interpreted with caution and a strong correlation between PET-scan, MRI data, and clinical findings is mandatory. To our knowledge, no patient with *PIGA* deficiency underwent neurosurgical procedure. In patient #1, the diagnosis of EIMFS was suspected because of multifocal seizures. EIMFS is mainly linked to gain of function mutations in the *KCNT1* gene, but sporadic cases are linked to other genes, including *KCNQ2*, *SCN2A*, *GABARG2*, *GABARB3*, *SLC12A5*, *SMC1A*, or *FGF12* [13]. We underline that *PIGA* mutations can be a differential diagnosis of this condition.

PIGA deficiency may also mimic a metabolic disease, including maple syrup disease, where MRI may reveal restricted diffusion in the brainstem tegmentum or the subthalamus and cerebellar peduncles. These areas are myelinated early in newborns and are likely to suffer from energetic defect [12].

A mitochondrial dysfunction is also possibly a consequence of deficient GPI-anchor proteins. In our series, a mitochondrial dysfunction was noted for patient #1, with abnormal biochemical tests evocative of complex-IV deficiency. Patient #5 had radiological features evocative of Leigh syndrome. The patient reported by Van der Crabben had abnormal overall production of ATP without any specific deficiency [14] and Swoboda reported two post-mortem studies with disorganized mitochondria in one case and cystic areas in the cortex and scattered normal neurons in the other, which were evocative of mitochondrial defect [2]. One of the two brothers reported by Joshi had features of increased lipid accumulation on muscle biopsy suggestive of a primary mitochondrial myopathy [5]. Owing to its wide clinical and biological heterogeneity, mitochondrial dysfunction is one of the main differential diagnoses of *PIGA* mutations, and is probably linked to the pathophysiology of epilepsy and developmental delay. Further research is needed to elucidate this issue.

Treatment in germline *PIGA* mutations remains challenging. The condition is severe and supportive care includes gastrostomy feeding and physiotherapy. Antiepileptic treatment is the cornerstone of treatment in this disorder, even if the best association of medications for early-onset *PIGA* epileptic encephalopathy has yet to be defined. Valproate, vigabatrin, and carbamazepine/oxcarbazepine were the most frequently used drugs in our series. Status epilepticus are frequent and require management in intensive care unit. However, periods of remission, with very few seizures, are also possible. A patient in the series reported by Kato (c.230G>T p.Arg77Lys) was seizure-free at age 3 years while treated with topiramate [9]. A ketogenic diet is a widely available treatment that may improve various epileptic conditions. A recent report by Joshi *et al.* underlined the efficacy of ketogenic diet in *PIGA* deficiency [5], but another study concluded in the contrary [15]. We did not observe any

dramatic response but in two cases a better quality of relation was present after diet implementation. Vagal nerve stimulation was tried for patient #1, with a transient reduction in seizure frequency. To our knowledge this is the first attempt of vagal nerve stimulation in *PIGA* deficiency, and so its benefit needs confirmation by other studies.

Sharp genotype-phenotype correlation has yet to be established. The five mutations described herein are only missense mutations. As mentioned above, the nonsense mutation c.1234C>T p.Arg412* leads to the most severe phenotype, including neonatal seizures with myoclonic epilepsy, burst suppression on EEGs, and early death [1, 9]. Kato in 2014 and Tarailo-Graovac in 2015 classified the *PIGA* mutation phenotypes between “severe” and “less severe” forms [9, 12]. The “severe” form comprises dysmorphic features, multi-organ involvement, joint contractures, elevated alkaline phosphatases, multiple central nervous system abnormalities and an often fatal outcome. Less severe forms are represented by patients exhibiting treatable epilepsy, without dysmorphism and a longer lifespan. The authors advocated that some anomalies are probably allele-specific. Patients #1-3 do not belong to the “severe” category, since none of them had either visceral malformations or marked dysmorphic signs. However, one of them is deceased and the two others suffer from intractable epilepsy with spastic tetraparesis. Patient #4 harbored the already published c.356 G>A mutation, that was considered as “severe” by the authors [16]. His clinical picture is very similar including developmental delay, infantile spasms at age 2 months, oculoclonic seizures, elevated alkaline phosphatases, and intractable epilepsy. Patient #5 is aged 3 and his epilepsy is currently controlled by two medications, so he has the “less severe” form of *PIGA* deficiency.

To date, the number of reported patients with *PIGA* deficiency is low. Except for some mutations, it is not yet possible to establish an accurate genotype-phenotype correlation. However, we believe that the phenotypic dichotomy between “severe” and “less severe” is probably too restrictive. Our patients with the so-called “less severe” conditions have actually a poor quality of life, including severe developmental delay and intractable epilepsy. Moreover, new mutations are regularly described, and *PIGA* mutations obviously lead to a wider range of severity than initially supposed. A classification in type1/2/3 might be more appropriate. Type 1 would comprise very early onset epilepsy (*i.e.*, in the first month of life), with visceral involvement and severe dysmorphic signs. Type 2 include early onset epilepsy (*i.e.*, in the first year of life) with intractable seizures and more subtle dysmorphic signs, without visceral involvement. Type 3 concern patients with controlled epilepsy with developmental delay but without dysmorphic signs. Further reports are mandatory to increase number of patients affected by *PIGA* deficiency, in order to permit early prognosis.

7. Conclusion

We have found four new mutations and widened the phenotype in germline *PIGA* deficiency. The differential diagnosis may be challenging when dysmorphic signs are subtle. At onset of epilepsy, a normal interictal EEG may be present. Early diagnosis of *PIGA* deficiency is of the utmost importance, in order to prevent unnecessary investigations that may be harmful and costly. Widespread use of whole exome sequencing is mandatory to correctly delineate this condition. Less severe phenotypes are probably under recognized at present. Physiopathology must be addressed, notably concerning the mitochondrial defect which seems to play an important role in the cellular dysfunction of these patients. Given the severity of epilepsy, new molecules and research protocols should be easily proposed for these patients.

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9. Figure captions

9.1 Figure 1. Photos of patients. A-B: patient #1. C: patient #2. D-E: patient #3. F-G: patient #4.

9.2 Figure 2. Electroencephalographic features. Top line: patient #4, age 6 months. Asymmetrical brain activity with right centro-temporal spike-waves, and slow rhythmic activity. Middle line: patient #1, age 3 years (left and middle) and 4 years (right): disorganized electrogenesis with diffuse spike-waves and global slowing. Bottom line : patient #2, age 1 year (left, onset of seizures) with hypersarhythmia and 2 years (middle and right) : bilateral centro-temporal spike waves (middle) ; fast rhythmic activity mainly in bilateral central areas with generalized seizure (right).

9.3 Figure 3. Neuroimaging features. A-B: Patient #4, 7 months. Widening of pericerebral spaces linked to cortical atrophy with slightly enlarged ventricles (A, T1-weighted image, B, T2-weighted image) C: Patient #1, 20 months. Delayed myelinisation in sustentorial zones (FLAIR), D : Patient #2, age 9 months: normal MRI on T1-weighted sagittal image. E-F: patient #5. Restricted apparent diffusion coefficient on thalami and pallidae (E) and on peri-aqueducal zone (F).

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figure 2.

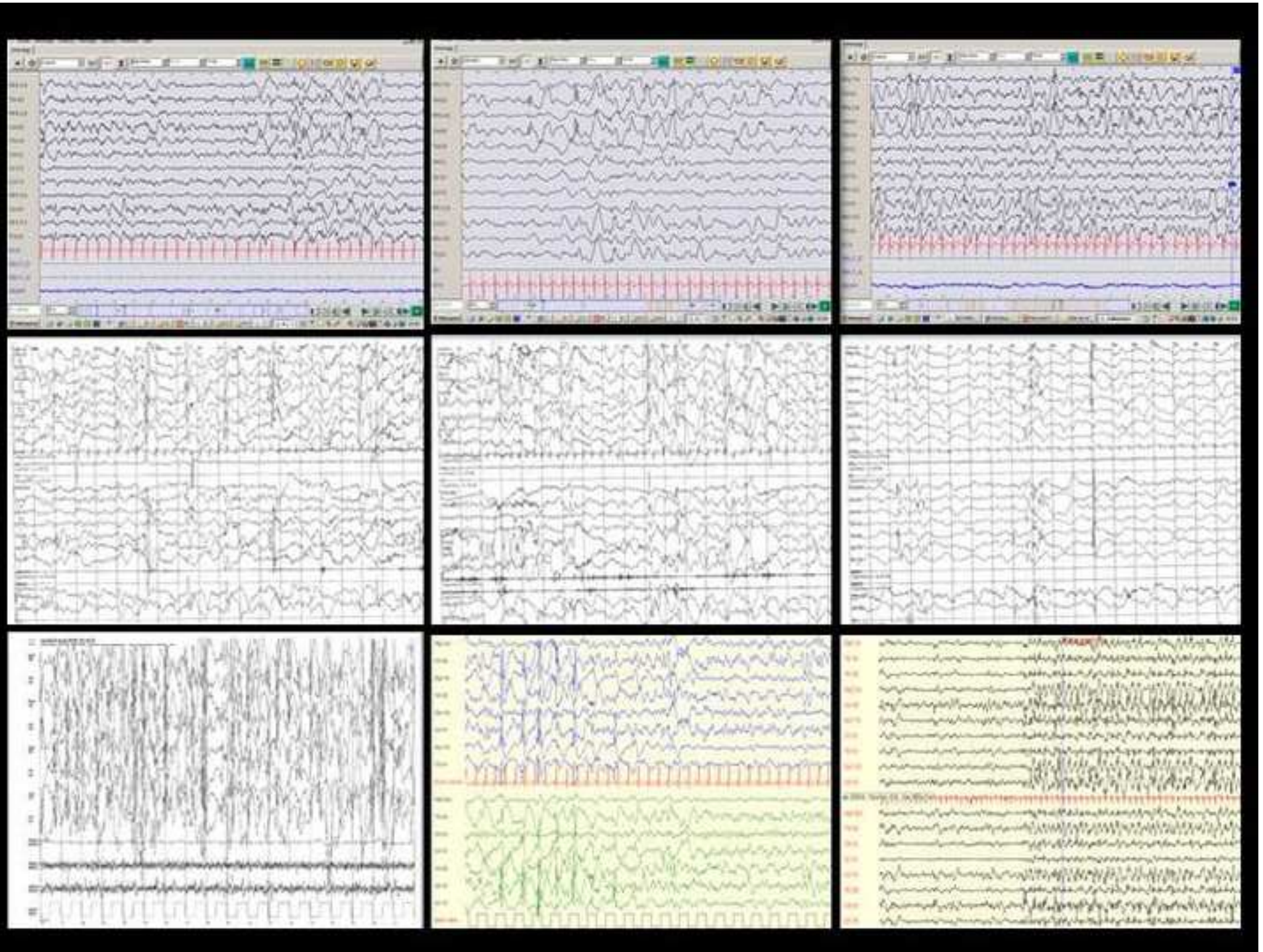


figure 3

