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Original article

Electroclinical characteristics of seizures—comparing Prader-Willi syndrome with Angelman syndrome☆

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Abstract

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are two clinically distinct neurobehavioral syndromes that are caused by deficiency of gene expression from paternally or maternally derived homologues on chromosome 15q11–q13, respectively. Clinical and genetic heterogeneities are common in both syndromes and they are now regarded as 'sister genetic imprinting syndromes'. This study aimed to describe and compare the electroclinical characteristics of seizures between PWS and AS, and to try to explore the possible mechanisms of epileptogenesis in these two syndromes. Fifty patients with genetically documented PWS and 18 patients with a putative diagnosis of AS were included in this study. These patients were diagnosed on the basis of characteristic physical findings and their neurobehavioral phenotype, as well as cytogenetic and molecular studies. Epileptic seizures were present in 16 of 18 patients with AS, but in only eight of 50 patients with PWS. Using electroencephalography (EEG), the most characteristic findings for AS were rhythmic 2–3 Hz delta waves of high-amplitude that were maximal over the frontal regions, and 3–4 Hz spikes and sharp wave runs posteriorly. These were never seen in PWS. Patients with AS had a much higher incidence of seizures with characteristic EEG findings, similar to those seen in mice that are deficient in a single gene (*UBE3A*) that displays regional brain-specific imprinting in humans and mice. In this series, cases with no detectable cytogenetic or molecular defect at the AS locus displayed similar AS phenotype, seizure severity and EEG abnormalities compared to those with such a defect. Thus, the *UBE3A* gene is presumed to be potentially involved in the epileptogenesis of AS. It is also possible that *UBE3A* and another gene located nearby, γ-aminobutyric receptorβ3 subunit, may interact in some way, and result in the severe epilepsy seen with AS. Some patients with PWS and AS share the common EEG features of persistent high-amplitude 4–6 Hz activity in recordings during sleep, and while awake. The significance of such EEG findings needs further experience to clarity.

Keywords: Prader-Willi syndrome; Angelman syndrome; Genetic imprinting; Epileptic seizures; Electroencephalography

1. Introduction

Prader-Willi syndrome (PWS [MIM 176270]) and Angelman syndrome (AS [MIM 105830]) are two clinically distinct neurobehavioral disorders that are nevertheless now regarded as 'sister genetic imprinting syndromes', because they share the same chromosomal abnormalities of 15q11–q13 [1–3]. PWS was first described in 1956 [4] and is characterized by grossly diminished fetal activity, hypotonia, and feeding problems in early infancy, followed by hyperphagia and subsequent central obesity in childhood. Patients also display hypogonadism or hypogenitalism, short stature, small hands and feet and psychomotor retardation. In addition, there are characteristic facial dysmorphisms including almond-shaped palpebral fissures, a narrow bifrontal diameter and downturned mouth, as well as behavioral problems and a tendeny to develop diabetes in adolescence [4–6]. AS was first described by Angelman in 1965 [7], and is characterized by severe mental retardation, inappropriate laughter, happy disposition, ataxic gait, jerky movements, lack of speech and dysmorphic craniofacial features. The diagnosis of AS is primarily a clinical one that

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can be confirmed by laboratory tests [8]. Epileptic seizures occur in most children with AS, but are rare in patients with PWS [2,9–12]. The purpose of this study was to describe and compare the electroclinical characteristics of seizures between PWS and AS.

2. Methods

2.1. Patients

Fifty patients with genetically documented PWS and 18 patients with a putative diagnosis of AS were enrolled in this study. Clinical evaluation of PWS was performed using the diagnostic criteria of Bulter and Holm et al. [5,6], while AS was evaluated using the criteria of Williams et al. [8]. Genetic studies including high-resolution G-banding, fluorescence in situ hybridization (FISH) and DNA methylation patterning were performed according to the methods of Williams et al. [8].

2.2. Cytogenetics

Peripheral blood lymphocytes (PBL) were cultured using standard procedures. High-resolution chromosome banding analyses were performed using methods that increase the frequency of prometaphase chromosomes [13]. Complete karyotype analysis of G-banded chromosomes (using standard trypsin-Giemsa staining techniques) was performed with close scrutiny for deletions of chromosome 15. In cases in which a deletion was not detected, clinical reassessment determined whether FISH was warranted. Patients with typical presentations of AS or PWS who had equivocal cytogenetic studies were subsequently investigated using FISH.

2.3. Fluorescence in situ hybridization (FISH)

FISH was performed using four PWS/AS cosmid probes that were obtained from Oncor Inc. (Gaithersburg, MD, USA): D15S11, small nuclear ribonucleoprotein-associated peptide N (SNRPN), D15S10, and γ-aminobutyric receptorβ3 subunit (GABRB3). The 15q22 cosmid marker, *myl*, was also used (Oncor protocol). These cosmids (D15S11, SNRPN, D15S10 and GABRB3) hybridize to specific sequences in bands 15q11–q13, which include the PWS/AS critical region [14]. The proposed order of the probes on chromosome 15 is D15S11–SNRPN–D15S10–GABRB [14]. After post-hybridization washing in 2× SSC (300 mmol/l NaCl, 30 mmol/l sodium citrate, pH 7.0) at 72 °C for 5 min, visualization was achieved through a series of enzymatic conjugations by horseradish peroxidase precipitation [15], followed by light microscopy.

2.4. Methylation test

Genomic PBL-derived DNA from patients with no detectable deletion over 15q11–q13, from both cytogenetic and molecular studies, was digested with *Hin*d III/*Hpa* II, separated on 0.8% agarose gels, and analyzed by Southern blot hybridization with the probe PW71 (D15S63) according to the protocol of Dittrich et al. [16].

2.5. EEG studies

EEG was performed for all 18 AS cases and for 26 of 50 PWS cases (eight with seizures, 18 without seizures), both while patients were awake and during normal sleep. Four of these 26 PWS cases were also subjected to EEG-respiratogram polygraphy, because of sleep apnea.

3. Results

3.1. AS findings

Microdeletions in the region 15q11–q13 were identified by high-resolution cytogenetic analysis in 11 patients with AS (group I) (Table 1). No other structural rearrangements such as translocation, inversion, or duplication were detected in this region. FISH studies not only confirmed the 11 cytogenetic deletions, but also identified three other molecular deletions (group II) (Table 1). Four cases yielded no evidence of chromosomal abnormality in this region, at both the cytogenetic and molecular level (group III) (Table 1). Two of these four patients were sisters.

Epileptic seizures had occurred in 16 of 18 patients (89%) with AS (nine out of 11 in group I, all of three in group II and all of four patients in group III). Three patients first experienced febrile seizures in infancy. Other seizure types included atypical absence seizures in 10 cases, myoclonic seizures in nine, generalized tonic–clonic seizures in seven and atonic seizures in three cases (Table 2). All 18 patients with AS displayed EEG

Table 1
Eighteen Angelman syndrome cases diagnosed by cytogenetic and molecular studies

Group	FISH				Seizure cases
	S11	NRPN	S10	GABRB3	
I ($n = 11$)	−	−	−	−	$n = 9$
II ($n = 3$)					$n = 3$
1	+	−	−	−	
2	+	+	−	−	
3	+	−	−	−	
III ($n = 4$)	+	+	+	+	$n = 4$

I, High-resolution cytogenetic deleted group; II, high-resolution normal, FISH deleted group; III, no deletion/UPD/methylation abnormalities group; *n*, number; ' − ', deleted; ' + ', non-deleted by FISH.

Table 2
Seizure types in cases of Prader-Willi and Angelman syndromes

	Prader-Willi syndrome	Angelman syndrome
Number of cases	50	18
Cases with seizures	8 (14%)	16 (89%)
Seizure type		
Atypical absence	1	10
GTC[a]	7	7
Myoclonic seizures	0	9
Atonic seizures	0	3
Febrile seizures	1	3

[a] GTC, Generalized tonic–clonic seizures.

Table 3
EEG features of 18 cases of Angelman syndrome

EEG features	Number of cases
Bifrontally-dominanted high-amplitude 2–3 Hz slow and sharp waves	14
Persistent high-amplitude 4–6 Hz activities not related to drowsiness	6
Spike and sharp wave runs with high-amplitude 3–4 Hz activity posteriorly	11
Normal EEG	0

abnormalities (Table 3). The most characteristic EEG features of AS were:

1. prolonged runs of rhythmic 2–3 Hz activity (200–500 μV) often more prominent bifrontally, associated with ill-defined spikes or sharp waves in 14 cases; and
2. spikes mixed with 3–4 Hz components (>200 μV), mainly posteriorly, and facilitated by eye closures in 11 cases (Figs. 1 and 2).

Six cases had persistent rhythmic high-amplitude 4–6 Hz activities in recordings of patients both awake and asleep (Fig. 3). The EEG features described above appeared in isolation or in various combinations, either in the same EEG recordings or at different times in the same patients.

3.2. PWS findings

Microdeletion of the region 15q11–q13 was identified by high-resolution cytogenetic analysis in 36 of 50 PWS patients (group I) (Table 4). No other structural anomalies such as translocation, inversion or duplication were involved in this region. FISH using PWS/AS probes confirmed the presence of the deletions in the 36 cytogenetically identified patients, and facilitated diagnostic evaluation of five other molecular deletions in both prometaphases and interphases (group II) (Table 4). The shortest region of deletion overlap was defined by the gene for *SNRPN* (Table 4). Methylation analysis using the probe PW71 revealed nine cases with maternal uniparental disomy (UPD). In these cases, a maternal 6.6 kb region was present, but the paternal 4.7 kb region was absent in all samples (group III) (Table 4).

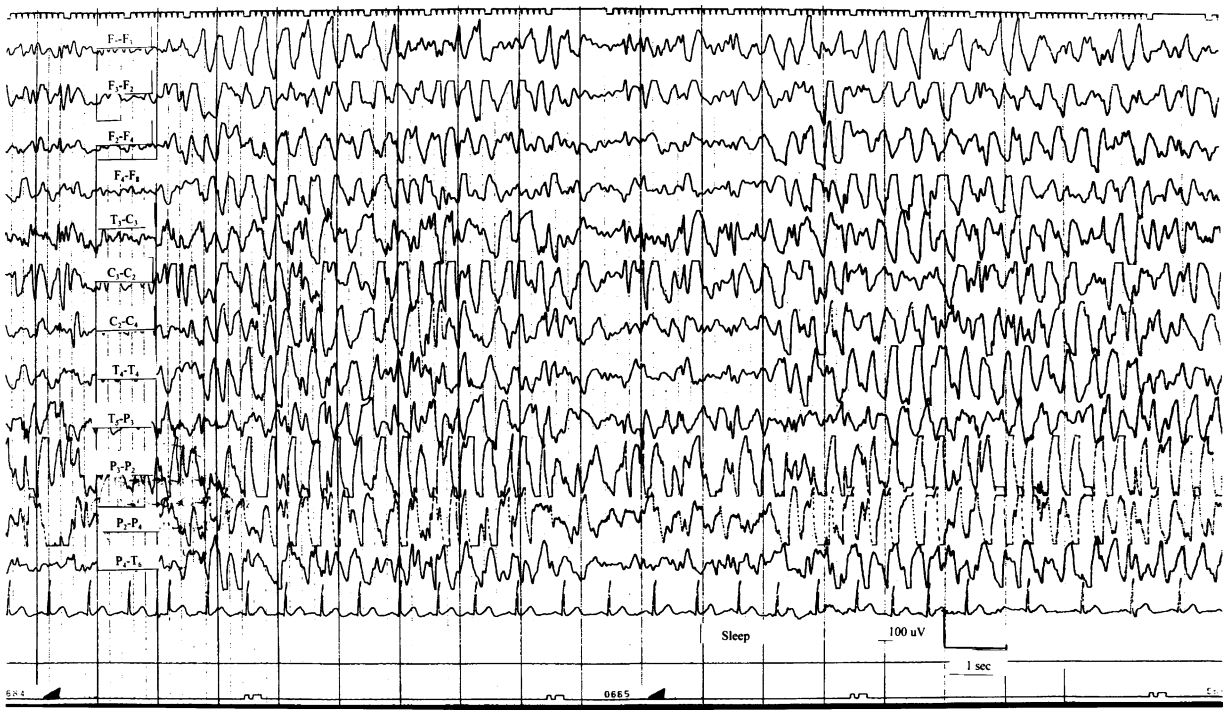

Fig. 1. Sleep EEG of a 5-year-old patient with AS with cytogenetically detected deletion of chromosome 15q11–q13. Recording shows bifrontally-dominanted high-amplitude 2–3 Hz slow and sharp waves, associated with posteriorly-dominanted high-amplitude 3–4 Hz spike and wave runs.

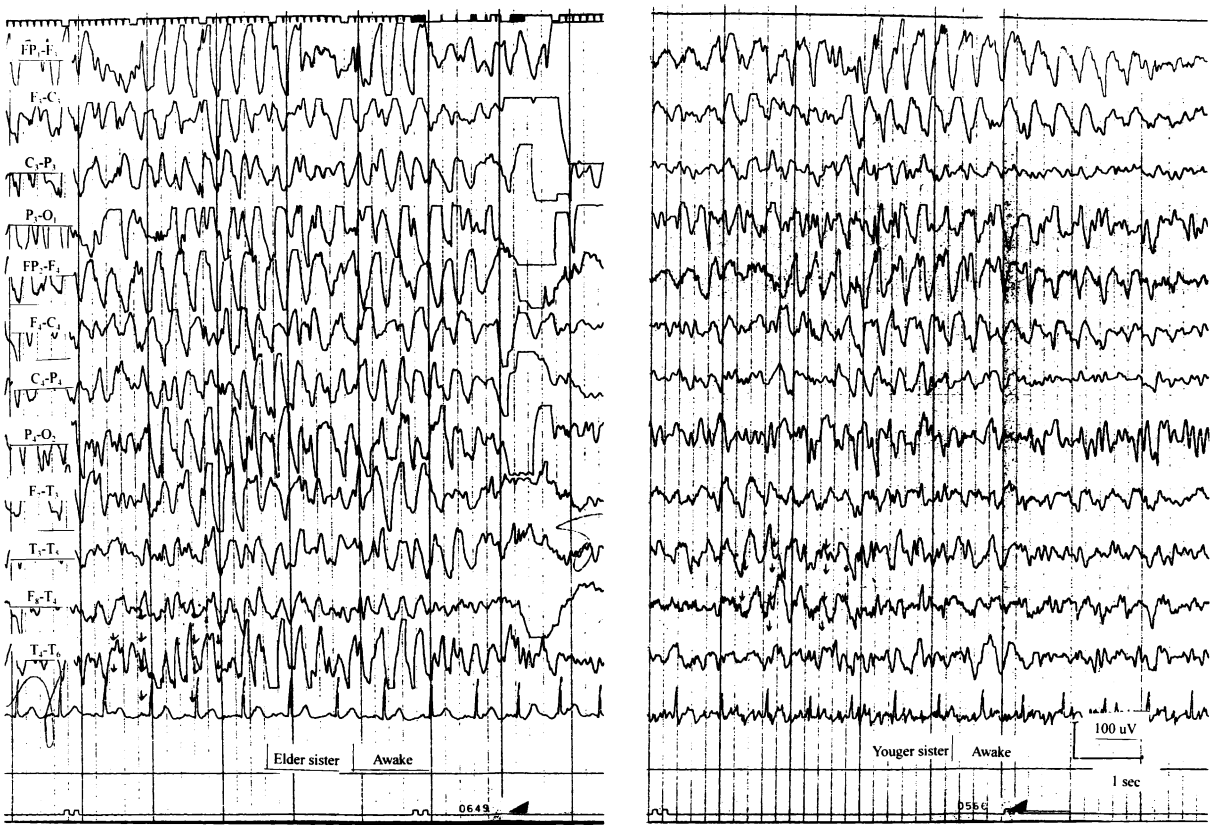

Fig. 2. Awake EEG in two sisters without cytogenetically or molecularly detectable deletions of the AS locus. Similar EEG features as seen in Fig. 1 (left: older sister, 11-year-old; right: younger sister, 9-year-old).

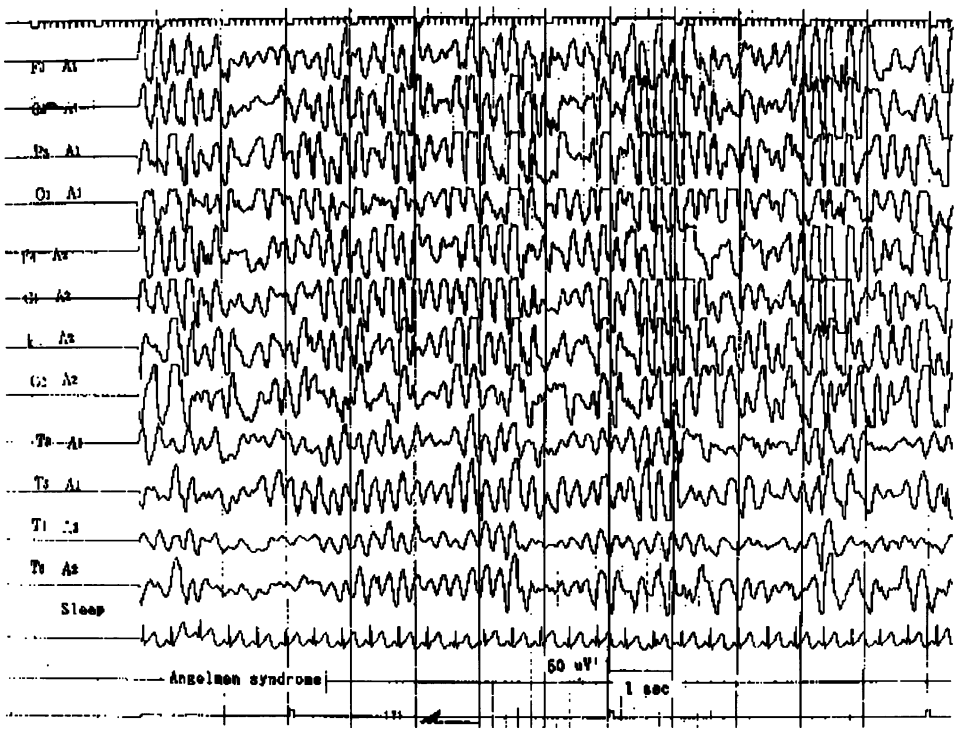

Fig. 3. Sleep EEG in a 6-year-old patient with AS with cytogenetically detected deletion of chromosome 15q11–q13. Persistent rhythmic 4–6 Hz activities reaching more than 200 μV are seen.

Table 4
Fifty cases of Prader-Willi syndrome diagnosed by cytogenetic and molecular studies

Group	FISH				Seizure cases
	S11	NRPN	S10	GABRB3	
I ($n = 36$)	−	−	−	−	$n = 8$
II ($n = 5$)					$n = 0$
1	−	−	−	−	
2	−	−	+	+	
3	+	−	−	−	
4	−	−	−	+	
5	−	−	−	+	
III ($n = 9$)	+	+	+	+	$n = 0$

I, High-resolution cytogenetic deleted group; II, high-resolution normal, FISH deleted group; III, maternal uniparental disomy group; *n*, number; ' − ', deleted; ' + ', non-deleted by FISH.

Eight of 50 patients (14%) with PWS had epileptic seizures. Seven of these had generalized tonic–clonic seizures, one of whom experienced initial febrile seizures. The other one patient was found to have atypical absence seizures (Table 2). All eight cases with clinical seizures belong to group I (those with cytogenetically detected deletions). EEG was performed in 26 of 50 patients with PWS and 10 of these 26 patients were noticed to have abnormal EEG findings. Five patients (both with and without seizures) had EEG features of persistent high-amplitude 4–6 Hz activities, from recordings both during sleep and while awake, resembling those of patients with AS (Fig. 4, Table 5). Focal paroxysmal discharges were seen in four patients with generalized tonic–clonic seizures. One patient with atypical absence seizures displayed short bursts of bilateral, synchronous polyspike and wave patterns on EEG (Fig. 5, Table 5).

4. Discussion

PWS and AS are distinct developmental and neurobehavioral syndromes that arise from abnormal imprinted-gene expression of the chromosomal region 15q11–q13 in humans. Around 70–80% of patients with PWS and AS have a large 4 Mb deletion in the paternally or maternally inherited 15q11–q13 region, respectively [17]. Both syndromes can also result from UPD, in which two maternal copies are inherited in patients with PWS and two paternal copies in patients with AS. UPD is more common in PWS than AS, because of higher rates of maternal non-dysjunction [18]. An unusual class of patients with PWS and AS (both $\leq 5\%$) has a mutation affecting the imprinting process [19,20], and a single gene (*UBE3A*) that displays regional brain-specific imprinting in humans and mice is implicated in AS [21,22]. In these cases, AS is associated with loss of maternal expression of the *UBE3A* gene. However, PWS is likely to be a contiguous-gene syndrome and, consistent with this hypothesis, multiple imprinted, paternally expressed genes have been identified in 15q11–q13 [17].

Epileptic seizures occur in around 80% of patients with AS [12,23,24]. A diversity of seizures is seen, which are often difficult to control, especially in early childhood. Genotype–phenotype correlation has been observed in

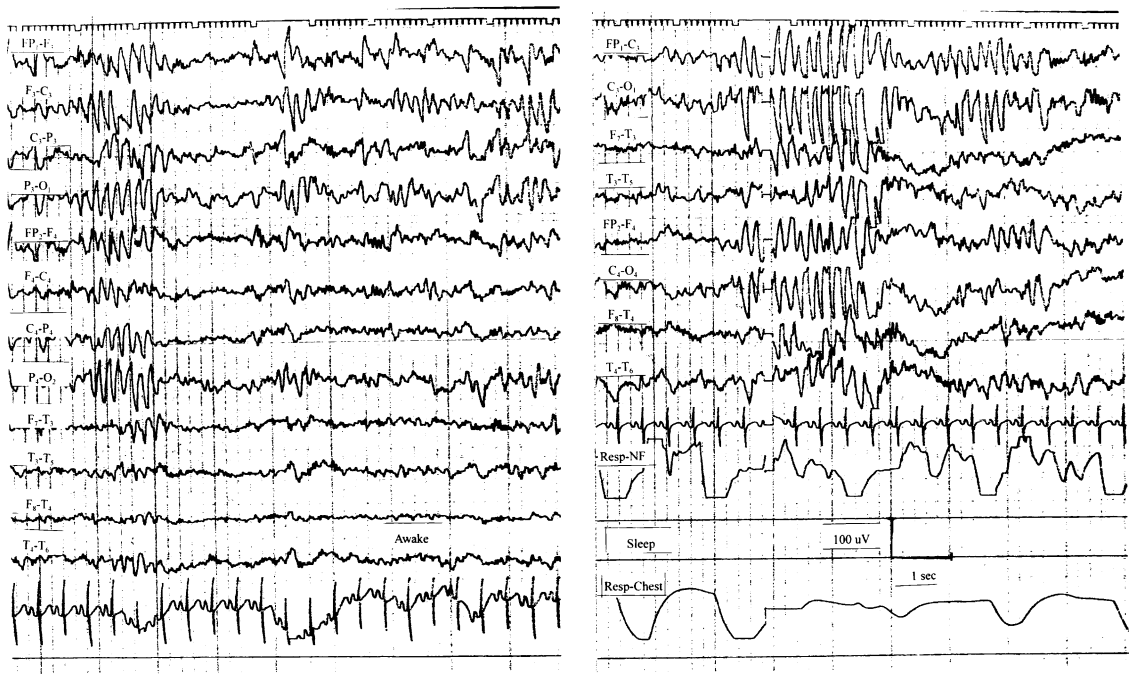

Fig. 4. EEG records in a 10-year-old patient with seizures and obstructive sleep apnea with cytogenetically proven PWS. Recordings show high-amplitude rhythmic, 4–6 Hz activities while awake (left) and during sleep (right).

Table 5
EEG features of 26 cases of Prader-Willi syndrome

EEG features	Number of cases
Persistent high-amplitude 4–6 Hz activities not related to drowsiness	5
Focal paroxysmal discharges	4
Polyspike and wave short bursts	1
Normal EEG	16

patients with AS [12,24], whereby patients with single *UBE3A* gene mutations, UPD or methylation imprinting abnormalities are associated with milder epilepsy than those with deletions. However, in our series, cases with no detectable cytogenetic or molecular defect at the AS locus displayed similar AS phenotype, seizure severity and EEG abnormalities compared to those with such a defect. The most characteristic EEG features of AS were bifrontally-dominated prolonged runs of rhythmic 2–3 Hz activity, combined with posteriorly-dominated 3–4 Hz slow and spike–waves. These EEG changes are diagnostically important in the appropriate clinical context, as they can appear before other clinical manifestations of AS [25,26]. Indeed, two of our patients aged younger than 9 months were identified with the aid of EEG findings, prior to the development of seizure activity.

The gene, *GABRB3*, which encodes the β3 subunit of the receptor to the inhibitory neurotransmitter γ-aminobutyric acid (GABA), is mapped to the AS region [27]. However, since patients with PWS also have large deletions at the same region, but only few have seizures or EEG abnormalities, and since 20–30% of patients with AS with epilepsy have no detectable deletion of 15q11–q13, deletion of *GABRB3* cannot be solely responsible for the associated EEG abnormalities and seizures in AS. Miura et al. [28] found that mice deficient in maternal *UBE3A* had intermittent bursts of 4–5 Hz spike–wave discharges lasting 5–12 s, and that both valproate and ethosuximide were effective in reducing such EEG abnormalities. AS cases have a much higher incidence of seizures with characteristic EEG findings, implicating a potential role for the *UBE3A* gene in the epileptogenesis of AS. It is also possible that the closely located *UBE3A* and *GABRB3* genes, may interact in some way, and result in the severe epilepsy of AS. Some PWS and AS cases share common EEG features of persistent high-amplitude 4–6 Hz activity in recordings from patients, both asleep and awake. The significance of such EEG findings need further experience to clarity.

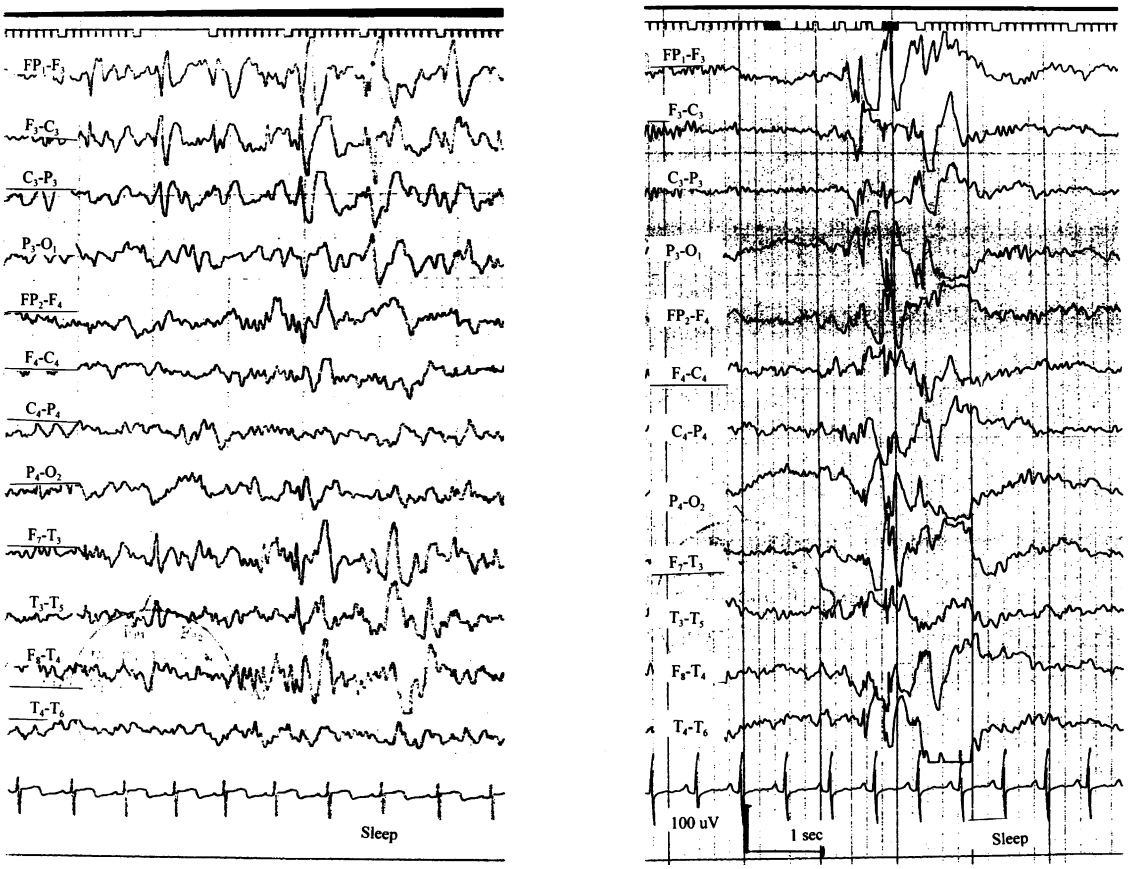

Fig. 5. Sleep EEGs in a 13-year-old and an 8-year-old with PWS. Focal paroxysmal spikes arising from the left frontal area in a 13-year-old with PWS (left), and bilateral, synchronous spike and wave short bursts in an 8-year-old with PWS with atypical absence seizures (right).

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