Clinical Neurophysiology 131 (2020) 1030–1039

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Expanding the clinical and EEG spectrum of *CNKSR2*-related encephalopathy with status epilepticus during slow sleep (ESES)

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ARTICLE INFO

Article history:
Accepted 13 January 2020
Available online 13 February 2020

Keywords:
CNKSR2
ESES
Encephalopathy
Speech/oro-motor dyspraxia
X-linked intellectual disabilities
Spike-wave-index (SWI)

HIGHLIGHTS

- Worsening of epilepsy associated with increment of awake spike-wave-index.
- A frontal topography of sleep EEG epileptic activity in the active phase of ESES.
- Language disorder due to speech/oro-motor dyspraxia.

ABSTRACT

Objective: To investigate the clinical and EEG features of Encephalopathy with Status Epilepticus during slow Sleep (ESES) related to *CNKSR2* pathogenic variants.
Methods: Detailed clinical history, repeated wakefulness/overnight sleep EEGs, brain MRI were collected in five patients, including one female, with *CNKSR2*-related ESES.
Results: Neurodevelopment in infancy was normal in two patients, delayed in three. Epilepsy onset (age range: 2–6 years) was associated with appearance or aggravation of cognitive impairment, language regression and/or behavioral disorders. Worsening of epilepsy and of cognitive/behavioral disturbances paralleled by enhancement of non-rapid eye movement (NREM) sleep-related, frontally predominant, EEG epileptic discharges [spike-wave-index (SWI): range 60–96%] was consistent with ESES. In three patients, episodes of absence status epilepticus or aggravation of atypical absences occurred, in this latter

Abbreviations: AED, anti-epileptic drugs; array-CGH, array-comparative genomic hybridization; EAS, epilepsia aphasia spectrum disorders; EEG, electroencephalogram; ESES, encephalopathy with status epilepticus during slow sleep; MRI, magnetic resonance imaging; NGS, next generation sequencing; NREM, non-rapid eye movement; REM, rapid eye movement; SWI, spike wave index; WES, whole exome sequencing; WISC-R, Wechsler intelligence scale for children revised; WPPSI, Wechsler preschool and primary scale of intelligence.

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https://doi.org/10.1016/j.clinph.2020.01.020

case associated with striking increment of awake SWI. Speech/oro-motor dyspraxia was diagnosed in four patients. In two patients, long-term follow-up showed epilepsy remission and persistence of mild/-moderate cognitive disorders and behavioral disturbances into adulthood.

Conclusions: Novel findings of our study are occurrence also in females, normal neurodevelopment before epilepsy onset, epilepsy aggravation associated with enhanced awake SWI, mild/moderate evolution in adulthood and language disorder due to speech/oro-motor dyspraxia.

Significance: Our findings expand the phenotypic spectrum of *CNKSR2*-related ESES.

1. Introduction

Encephalopathy with status epilepticus during slow sleep (ESES) (Tassinari et al., 1977) is a self-limiting, age-related, epilepsy syndrome characterized by different seizure types, neuropsychological regression, behavioral disorders and a typical electroencephalogram (EEG) pattern of extreme activation of epileptic discharges during non-rapid eye movement (NREM) sleep (Patry et al., 1971). Recently, pathogenic variants in several genes, including *GRIN2A* and *CNKSR2*, have been reported to be associated with epilepsia-aphasia-spectrum disorders (EAS), a spectrum of conditions that encompasses ESES (Kessi et al., 2018; Lesca et al., 2019). So far pathogenic variants in the *CNKSR2* gene have been reported only in few patients with ESES (Lesca et al., 2012; Vaags et al., 2014; Damiano et al., 2017; Sun et al, 2018). Few additional adult patients in whom ESES was speculated to have occurred in childhood, have also been described (Vaags et al., 2014; Aypar et al., 2015; Hu et al., 2016; Damiano et al., 2017).

CNKSR2 gene (OMIM *300724), located on chromosome Xp22.12 encodes a multidomain synaptic scaffold and adaptor protein, connector enhancer of KSR2. The protein is exclusively expressed in the brain, mostly in the hippocampus, amygdala, caudate nucleus and cerebellum (Nagase et al. 1998). It connects with densin-180, PSD95, and S-SCAM in the neuronal postsynaptic density (PSD) (Yao et al. 2000, Ohtakara et al. 2002) and participates in the downstream pathway of RAS-MAPK signal transduction (Therrien et al., 1998, Lanigan et al. 2003, Wellbrock et al., 2004) regulating neuronal complexity and synaptogenesis (Lanigan et al. 2003, Hu et al. 2016) as well as neuronal proliferation, migration, differentiation and death (Bumeister et al. 2004, Liu et al. 2009). Study on *CNKSR2*-depleted animals, in addition, demonstrated a reduction of the complexity and length of hippocampal neurons (Hu et al. 2016) confirming the possible role in synaptogenesis.

Here we report the clinical and EEG findings of five novel patients with *CNKSR2*-related ESES, aiming to expand the phenotypic spectrum of this rare disorder.

2. Material and methods

Five patients with ESES associated with *CNKSR2* pathogenic variants were collected through data sharing with European Epilepsy and Genetic Centers. All patients underwent brain magnetic resonance imaging (MRI) scanning and repeated wakefulness/overnight sleep EEG recordings. The duration of the wakefulness EEGs could vary from 20 to 60 minutes, whereas the duration of the wakefulness/overnight EEG could vary from 12 to 18 hours in the different centers. During the course of the disease, sleep EEGs during daytime including a full sleep cycle were also performed, usually to evaluate response to treatment.

Array-Comparative Genomic Hybridization (array-CGH), targeted gene panel sequencing performed by Next Generation Sequencing (NGS) or whole exome sequencing (WES) were performed as part of the formal diagnostic work-up in all patients. Written informed consent from parents/legal guardians and approval from the local ethical committees were obtained.

2.1. EEG recording and analysis

EEG signals were recorded through 19 silver-silver chloride electrodes placed over the scalp according to the International 10–20/10–10 system. In patient #1, data were exported to .edf (European data format) and further processed with MATLAB (The Math Works Inc., Natick, MA). An anti-aliasing low-pass FIR filter was applied and downsampling to 128 Hz was performed. Sleep stage scoring was based on 20 second epochs. Due to the extremely altered sleep-EEG pattern, sleep stage scoring was simplified in "wakefulness", "NREM-sleep" and "REM-like sleep", as in previous studies (Bölsterli et al., 2017, Pavlidis et al, 2019). Epochs disturbed by artifacts were eliminated. Spike amplitude and density, and electrode site showing spike activity leading to secondary bilateral synchrony were the criteria used to identify the EEG epileptic focus. A semi-automated spike search method implemented in the BESA software (BESA® Research 6.0) was used to calculate the spike-wave-index (SWI). Spikes were detected by template matching, in which an average of the visually identified, representative spikes for each EEG served as template. A 4–40 Hz zero phase band pass filter was applied to minimize the possible influence of sleep slow waves on the accuracy of template matching (Larsson et al., 2009). A virtual average montage over all channels was used for spike search with a correlation percentage of 80%. Calculation and graphical representation of the SWI was performed in a MATLAB environment (MATLAB, version 7.3.0 R2006b). In patients #3 and #4, SWI was assessed visually by expert neurophysiologists on paper-EEG; in these patients, SWI was assessed by calculating the amount of NREM sleep (in seconds) occupied by epileptic discharges divided by the total time of NREM sleep (in seconds) and then the ratio was transformed in percentage. In patients #2 and #5, a similar method was used on digital-EEG.

3. Results – Electro-clinical phenotyping (Table 1)

3.1. Patient 1

This Danish 5-years 8-months-old boy was born after an uneventful pregnancy, second child of healthy unrelated parents. He presented with breastfeeding problems and difficulty in swallowing since the first months of age. Developmental delay was first noticed at 5 months of age. When he was 20 months old, neurological examination showed poor language, gross motor function delay, poor balance due to diffuse hypotonia, joint hypermobility, and impaired walking, possible only with support. He had adequate fine motor function and he could manipulate toys with good coordination, using both hands with pincher grasp. His language consisted of several different words with meaning and he could pronounce two-word sentences; language understanding was overall good. At the age of 2 years, he started to suffer from nocturnal epileptic seizures, characterized by staring, complex motor manifestations and sometimes vomiting, often related to fever. Epilepsy was diagnosed at the age of 3 years 6 months, after the appearance of diurnal seizures, characterized by slurred speech without impairment of consciousness. Awake EEG disclosed multifocal 3–4 Hz spike-waves discharges. At this age, genetic tests (array-CGH, X-fragile) and metabolic screening were negative. Brain MRI was normal. At 3 years 7 months of age, fine motor functions worsened; in addition, he was diagnosed with speech and oro-motor dyspraxia. In the following years, physiotherapy, speech education and occupational therapy improved motor skills and language. At the age of 5 years, the detection of diffuse, continuous spikes/sharp waves at 2–2.5 Hz, with bilateral fronto-centro-parietal predominance, during NREM sleep coupled with seizure aggravation, worsening of motor coordination, cognitive regression, lack of eye contact, and complete loss of speech and language comprehension led to the diagnosis of ESES. SWI was 28% during wakefulness, 93% and 56% during NREM and REM sleep respectively (Fig. 1A). Several anti-epileptic drugs (AEDs) failed to achieve seizure control; a course of oral prednisolone worsened impulsivity and hyperactivity. Four months later, a wakefulness/overnight EEG (Fig. 1B) confirmed the extreme activation of the fronto-central spike/polyspikes-and-wave discharges during sleep and it showed an increment of the epileptic abnormalities during wakefulness: SWI was 44% during wakefulness, 95% and 47% during NREM and REM sleep respectively. At the last follow-up at the age of 5 years 6 months, he had regained eye contact; fine motor skills and cognitive status were mildly improved with partial recovery of expressive language (mainly echolalia). Hypotonia, hyperkinetic behavior, apraxic gait and speech/oro-motor dyspraxia were unchanged. In addition, he presented with daily multiple atypical absences, with staring and arrest of ongoing activities up to 40–45 seconds. A wakefulness/overnight EEG showed a striking enhancement of the epileptic discharges during wakefulness with awake SWI up to 90%; NREM-SWI was 96% (Fig. 1C).

At 5 years of age, genetic analysis, performed by NGS with a targeted panel for 582 genes associated with epilepsy, intellectual disability or autism spectrum disorder, disclosed a novel *de novo* hemizygous mutation of *CNKSR2*, c.2024_2027delAGAG leading to p.(Glu675Glyfs*41) on exon 18.

3.2. Patient 2

This Spanish 21-year-old man, second child of healthy unrelated parents, was born after an uneventful pregnancy. He had a normal psychomotor development until the age of 3 years. At this age, he presented his first seizure characterized by a prolonged episode (1 hour) of unresponsiveness and upward eyes deviation. One month later, he started to suffer from seizures characterized by stiffening of the body followed by tonic-clonic manifestations, occurring during sleep or in the sleep/wakefulness transition. In the post-ictal recovery phase, an expressive aphasia was evident. EEGs showed abundant 2.5 Hz spike-wave discharges in the frontal regions, with right side predominance, both during wakefulness and sleep. After seizure onset, he started to present with hyperactive behavior. At 6 years of age, worsening of hyperactivity associated with a remarkable enhancement of epileptiform discharges with frontal predominance during NREM sleep (NREM-SWI > 90%) (Fig. 2), led to the diagnosis of ESES. Brain MRI was normal. At 8 years of age a neuropsychological evaluation showed an intelligent quotient (IQ) in the low average range with attention deficit possibly related to medications, poor visuomotor coordination with normal verbal/auditory memory and an estimated age of 5 years. Sleep-related seizures continued uncontrolled despite different combinations of eight different AEDs, corticosteroids and adrenocorticotropic hormone (ACTH) treatment until the age of 12 years. At this age, seizures disappeared; EEG normalized by the age of 13 years. At present, he is seizure free and out of medications; he still has hyperactive behaviors and a mild cognitive impairment, with reading and writing difficulties, despite logopedic treatment.

At 16 years of age, genetic testing by WES disclosed a novel variant of *CNKSR2* gene, c.246-247delAG, leading to a frameshift p.T83Kfs*30. Parents were asymptomatic and tested negative for the mutation.

3.3. Patient 3

This French 12-year-old boy, second child (out of three) of healthy unrelated parents, was born after an uneventful pregnancy. Mild developmental delay was diagnosed soon after birth. He was able to sit at 10 months of age and to walk without support at 28 months of age. He also presented with stereotypies and a language disorder that was diagnosed as speech dyspraxia. Myoclonic seizures during sleep/wakefulness transition appeared at the age of 2 years 6 months. Epilepsy was later diagnosed at 4 years of age after the appearance of clonic seizures. At the age of 7 years, he started to present also atypical absences with head drops. EEG at epilepsy onset showed poorly organized background activity with abundant bilateral fronto-temporal epileptiform discharges. At 5 years of age, his language was severely impaired (he could pronounce only single words). Brain MRI at this age was normal. At 6 years 6 months of age, worsening of behavior associated with the appearance of continuous, bilateral fronto-temporal spike-waves during sleep (NREM-SWI > 80%) (Fig. 3), was consistent with the diagnosis of ESES. Language further regressed at the age of 8 years. Two prolonged episodes of absence status epilepticus occurred at the age of 7 years 6 months and 11 years, respectively. At present, he still suffers from uncontrolled seizures. At the last follow-up at the age of 12 years, he had moderate/severe neurodevelopmental delay (neuropsychological testing was not possible due to lack of cooperation) and his language was severely impaired (he could pronounce about 20 single words), with minimal improvement after speech therapy. Sleep EEG showed an overt decrease of epileptic discharges during NREM sleep, compatible with the remission of ESES. A trio-based WES, at 12 years of age, disclosed a novel deletion on chromosome X (c.457_461del) of *CNKSR2* gene leading to a pathogenic frameshift variant (p.Tyr153-Serfs*5). Segregation analysis of the parents disclosed a maternal mosaicism (5/156 reads) in the asymptomatic mother.

3.4. Patient 4

This French 41-years-old woman is the only child of healthy unrelated parents. She normally developed until 6 years 3 months of age, when she started suffering from tonic-clonic seizures. A treatment with valproic acid was initiated. At the age of 7 years, atypical absences with falls and cognitive disturbances such as difficulties in reading, writing and understanding complex instructions appeared. On Wechsler Preschool and Primary Scale of Intelligence (WPPSI) testing, she had a full-scale IQ of 62, verbal IQ of 66 and performance IQ of 57. Awake EEG showed generalized spike-wave discharges and right temporal spikes. At 8 years of age, complete loss of language, apraxia and unresponsiveness to external noises and episodes of absence status epilepticus were reported. Moreover, she could alternate periods of apathy with periods of hyperactivity and compulsive behavior. At this age, a striking activation of bilateral spike-and-wave discharges during sleep (NREM-SWI > 60%) led to the diagnosis of ESES. Brain MRI was normal. At

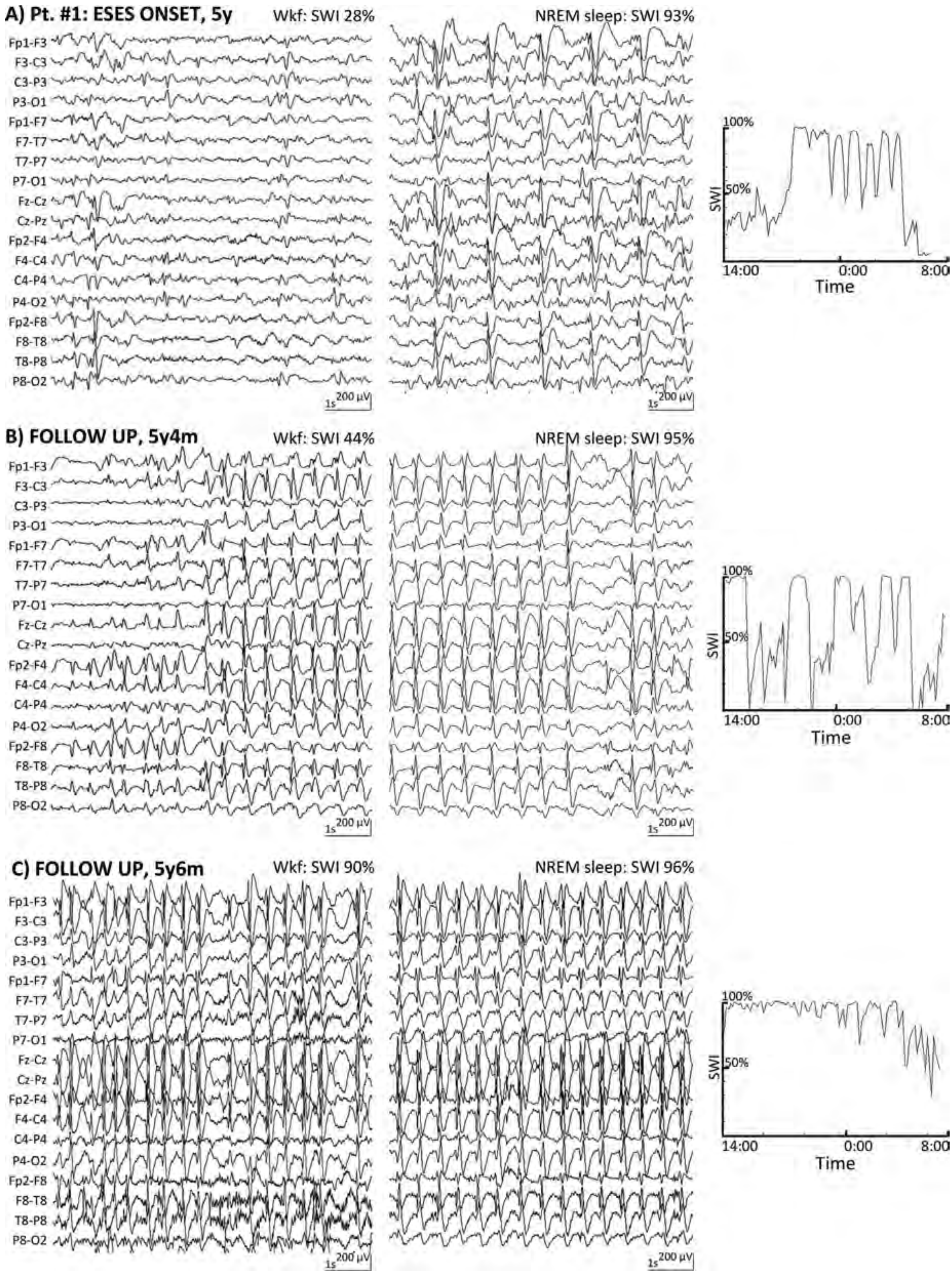

Fig. 1. Wakefulness and NREM sleep EEG recording in patient #1. On the left EEG tracing in wakefulness and NREM sleep are shown. On the right, graphs illustrating the SWI in wakefulness and sleep are reported during 18-hour recording (start of recording: 2 p.m.; end of recording 8 a.m. the day after). (A) At ESES onset (age 5 years) wakefulness EEG showed multifocal bilateral spike/spike-waves discharges (wakefulness SWI: 28%). During NREM sleep, diffuse 2.5 Hz spike-wave activity, with predominance over the bilateral fronto-centro-parietal regions (NREM-SWI: 93%) appeared. (B) First follow-up (age 5 years, 4 months): during wakefulness, striking activation of 2.5 Hz spike-wave activity, predominant in fronto-central regions and on the right side (awake SWI: 44%). During sleep, the NREM-SWI is 95%. (C) At the last follow-up (age 5 years 6 months): epileptic activity during wakefulness was further enhanced (awake SWI: 90%) whereas during NREM sleep SWI was unchanged as compared to the previous recordings (NREM-SWI: 96%). The SWI was calculated by using a semi-automated spike search method implemented in the BESA software (BESA® Research 6.0) (Larsson et al., 2009). Legend: EEG: Electroencephalogram; ESES: encephalopathy related to status epilepticus during slow sleep; m: months; NREM: non-rapid eye movement sleep; Pt.: patient; SWI: spike-wave index; y: years; Wkf: wakefulness.

Table 1
Clinical, EEG and genetic features of our cohort and already published patients affected by *CNKSR2*-related ESES.

Patient	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Sun et al., 2018	Damiano et al., 2017*	Damiano et al., 2017*	Vaags et al., 2014**	Vaags et al., 2014**	Vaags et al., 2014
Gender/Age Origin	M / 5y8m Danish	M / 21y Spanish	M / 12y French	F / 41y French	M / 9y8m Spanish	M / 8y8m Chinese	M / 18y Ashkenazi Jews	M / 12y Ashkenazi Jews	M / 6y Canadian	M / 8y Canadian	M / 8y Norwegian
Mutation	c.2024_2027delAGAG, p.Glu675Glyfs*41	c.246-247delAG, p.T83Kfs*30	c.457_461del, p. Tyr153Serfs*5	Deletion Xp22.12 (21523673–21558329)# §	Deletion Xp22.12 (21609392–21619786) #	c.2185C > T, p. Arg729*	c.2314C > T, p. Arg712*	c.2314C > T, p.Arg712*	Deletion Xp22.12 (20,297,696–21,471,387)#	Deletion Xp22.12 (20,297,696–21,471,387)#	Deletion Xp22.12 (21,375,312–21,609,484)#
Occurrence	*De novo*	*De novo*	Maternal mosaicism (5/156 reads)	Parents unavailable	*De novo*	*De novo*	Maternal (unaffected)	Maternal (unaffected)	Maternal (unaffected)	Maternal (unaffected)	Maternal (unaffected)
Sz onset - age Sz type at onset	2y Sleep-related Sz with staring and complex motor behavior	3y Prolonged episode of unresponsiveness and upward eyes deviation	4y TCS	6y TCS	3y2m Staring with bilateral "tremors" of upper limbs	~ 2y Episodes of loss of consciousness, staring, limbs jerks	3y6m NA	3y6m NA	2y Sleep-related TCS	2y Sleep-related TCS	2y6m Staring spells
Other Sz types	Episodes of slurred speech without impairment of consciousness	Sleep-related Sz with body stiffening evolving to TCS	Myoclonic Sz, atypical absences with head drops, abs-SE	Atypical absences with falls, abs-SE	Loss of tone followed by confusion/ agitation. TCS, hemiclonic seizures +/- loss of awareness	No	NA	NA	No	TCS	TCS
Sz outcome	Multiple daily atypical absences	12y: seizure free	Atypical absences with head drops	12y: seizure free	9y8m: 1–2 Sz/y	Improvement	NA	NA	Seizure free	Seizure free	Seizure free
WKF-EEG	3y6m: multifocal SW	3y: spikes, SW in L-O and biFr regions	4y6m: normal. 3y: disorganized BGA, multifocal spikes, biFr-T SW	6y3m: R-T spikes and GSW	2y10m: normal BGA. triphasic high-voltage spikes (L Fr-C-T and R F-T region)	NA	NA	NA	NA	NA	NA
ESES onset – age NREM-SWI ESES topography	5y Last F-UP: 96% 5y: biFr 5y6m: diffuse	6y >90% biFr (R > L)	6y6m >80% biFr-T (L > R)	~ 8y >60% Diffuse (R > L)	2y10m >80% biFr-T (R > L)	NA NA biT and Fr	~ 4y NA biC-T or Fr	3y6m NA biC-T or Fr	2y 80–100% Fr-T	~ 3y >80% Fr-T	7y NA Fr-T
ESES evolution	Ongoing	13y: remission	12y: remission	12y: remission	9y8m: ongoing	9y8m: speech improvement. No EEG.	NA	NA	4y10m: NDD. EEG: SWI 90%	NA	NA
AEDs°°	OXC, VPA, OCS, **CLB, STM, LEV**	ETS, LTG, CBZ, OXC, LEV, PHT, VPA, CLB, OCS, ACTH	CLB, **VPA, LEV, ZNS, CNZ**	VPA, ETS, ICS, OCS	**VPA**, LEV, CLB, ETS, STM, ZNS, RUF, LTG, PER, LCS, OCS, KD	IgIV, OCS, LTG, **VPA, LEV**	NA	NA	VPA, DZP, OCS	LTG, CBZ, **VPA**	**LTG**
ND before Sz onset	Mild NDD. 20 m: hypotonia, gross motor delay, speech/ oro-motor dyspraxia, instability	Normal	Mild NDD. 10 m: sitting; 20 m: walk with support; 28 m: walk without support	Normal	Moderate NDD. 20 m: hypotonia, language delay	NDD, ADHD	NDD and language delay	NDD and language delay	Mild NDD	Mild NDD	Mild NDD

Table 1 (*continued*)

Patient	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Sun et al., 2018	Damiano et al., 2017[*]	Damiano et al., 2017[*]	Vaags et al., 2014[**]	Vaags et al., 2014[**]	Vaags et al., 2014
Cognition, language and behavior before ESES onset	Mild NDD. Hypotonia, gross motor delay, speech/oro-motor dyspraxia, instability. 3y6m: fine motor delay	Hyperactivity	Stereotypies, speech dyspraxia. 5y: 50 single words	Mild NDD. Difficulties in reading, writing, comprehension	Moderate NDD. 20 m: hypotonia, language delay	ND regression	NDD and language delay. Attention deficit, hyperactivity	NDD and language delay. Attention deficit, hyperactivity	Mild NDD	Language regression	6y: single words ADHD
Cognition, language and behavior after ESES onset	Worsening of motor coordination, cognitive regression, complete loss of speech and language comprehension, lack of eye contact	ND regression	Moderate/severe NDD. 8y: language regression, 20 single words	ND regression. 10y: dysarthria, aphasia, bucco-lingual-facial dyspraxia, hyperactivity 12y: WISC-R: FSIQ 40.	3y: language delay (3–5 words), dyslalia, mild orolingual dyspraxia	Further ND regression. No speech, ASD	4y: ND regression. No speech	12y: useful language, attendance at regular school	ND Regression. 5y: no speech. Hyperactivity	ND regression. 4y: no speech. Hyperactivity	Severe NDD, inattention, impulsivity
Long-term evolution after ESES remission	NA	Mild NDD. 21y: specific disorder of reading and writing. Hyperactivity	NA	29y: WISC-R: FSIQ 54. 41y: basic reading and mathematics. Family dependent. Limited social life	NA	NA	18y: institutionalized	NA	NA	NA	NA
MRI scanning	Normal (3y)	Normal (6y)	Normal (5y)	Normal (8y)	Normal (3y)	Normal	NA	NA	Normal	Normal	Normal

Legend: abs-SE: absence status epilepticus; ACTH: Adrenocorticotropic hormone; ADHD: attention deficit hyperactivity disorder; AEDs: anti-epileptic drugs; ASD: autism spectrum disorder; BGA: background activity; bi-: bilateral; C: central; CBZ: carbamazepine; CLB: clobazam; CNZ: clonazepam; DZP: diazepam; EEG: Electroencephalogram; ESES: encephalopathy related to status epilepticus during slow sleep; ETS: ethosuximide; F: female; Fr: frontal; FSIQ: full scale intelligence quotient; F-UP: follow-up; GSW: generalized spike-waves; ICS: intravenous corticosteroids; IgIV: intravenous immunoglobulin; KD: ketogenic diet; L: left; LCS: lacosamide; LEV: levetiracetam; LTG: lamotrigine; M: male; m: months; MRI: magnetic resonance imaging; NA: not available; ND: neurodevelopment; NDD: neurodevelopmental delay; NREM: non-rapid eye movement sleep; O: occipital; OCS: oral corticosteroids; OXC: oxcarbazepine; PER: perampanel; PHT: phenytoin; R: right; RUF: rufinamide; STM: sulthiame; SW: spike-waves; SWI: spike-wave index; Sz: seizures; T: temporal; TCS: tonic-clonic seizures; VPA: valproic acid; y: years; WISC-R: Wechsler Intelligence Scale for Children Revised; WKF: wakefulness; ZNS: zonisamide.

[*] Patients belong to the same family.

[**] Patients belong to the same family.

[#] Human Genome version 19 by UCSC genome browser.

[§] Genetic data already published by Lesca et al. (2012).

[°°] AEDs with reported best efficacy are underlined. Current AEDs at last available evaluation are highlighted in bold.

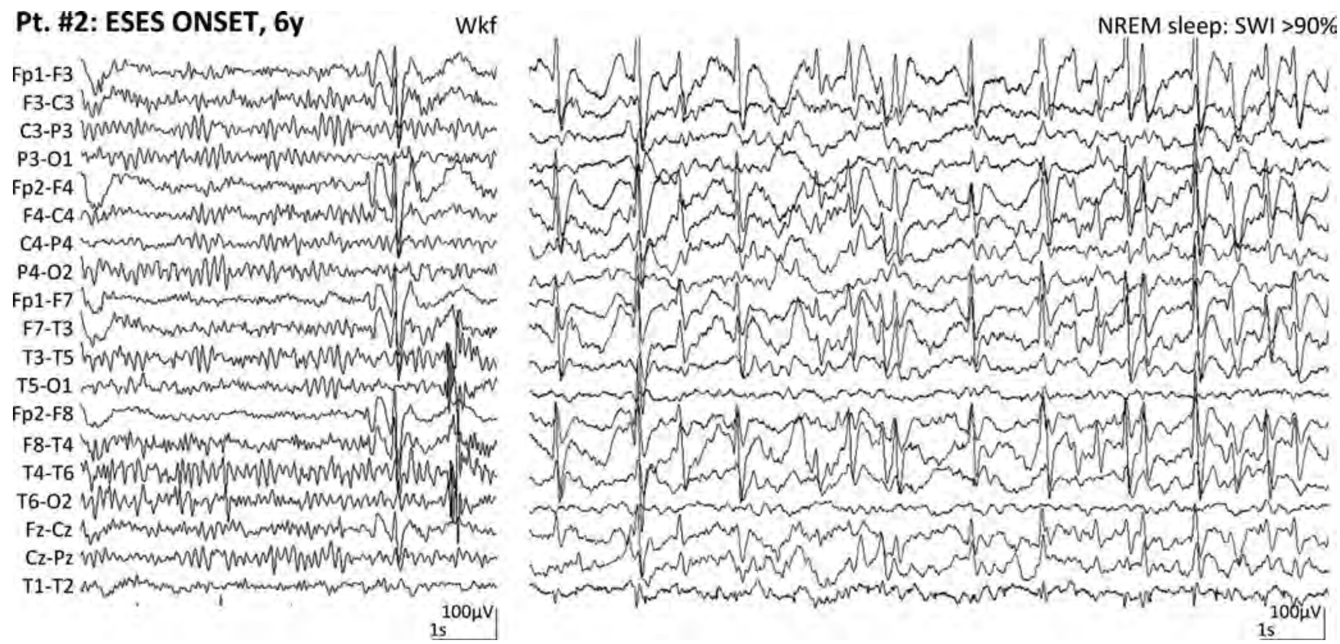

Fig. 2. Wakefulness and NREM sleep EEG recording in patient #2. At the age of 6 years, wakefulness EEG (left panel) showed sporadic bifrontal high amplitude spike-waves. During NREM sleep (right panel), exaggeration of bifrontal spike/spike-wave discharges with right side predominance (NREM-SWI: >90%). Legend: EEG: electroencephalogram; ESES: encephalopathy related to status epilepticus during slow sleep; NREM: non-rapid eye movement sleep; Pt.: patient; y: years; Wkf: wakefulness.

9 years of age, EEG showed frequent short bursts (1–2 sec) of generalized spike-and-waves during wakefulness and unchanged NREM-SWI. Short courses of intravenous hydrocortisone until the age of 10 years improved transitorily her cognitive status and behavior. EEG recording showed a decrement of epileptic abnormalities during wakefulness, whereas NREM-SWI was still around 60%. At 10 years of age, she further deteriorated presenting with dysarthria, oro-lingual-facial dyspraxia and severe aphasia. After a further cycle of prednisolone, her clinical status and EEG progressively improved. By the age of 11 years 11 months, the sleep EEG was normal; neuropsychological tests showed persistence of cognitive deficits (at Wechsler Intelligence Scale for Children Revised (WISC-R) testing: full-scale IQ 40, verbal IQ 49, performance IQ 45). No data on physiotherapy or rehabilitation are available. At age of 12 years 11 months, both awake and sleep EEG were normal. She is seizure free without medications since the age of 16 years. A neuropsychological assessment at the age of 29 years demonstrated moderate intellectual disability (at WISC-R testing: full-scale IQ 54, verbal IQ 60, performance IQ 52). At the last follow-up at the age of 41 years, she had very basic reading and mathematics abilities, and she was dependent on her family with limited social life. At the age of 34 years, an array-CGH disclosed a copy number change of 35 kb size on Xp22.12, ChrX:21523673-21558329 causing the loss of part of *CNKSR2* gene (genetic findings were previously published by Lesca et al., 2012). Parents were not available for testing.

3.5. Patient 5

This Spanish 9-years 8-months old boy was born at 36 gestational weeks from C-section after a bicorial biamniotic pregnancy. His mother suffered from epilepsy since the age of 18 years, controlled by antiepileptic treatment, and a borderline behavioural disorder. Soon after birth, mild generalized hypotonia became evident associated with unspecific developmental delay. He walked at 20 months of age; at this age, he presented with attention deficit, and a severe language delay, characterized by only few single words and mild oro-lingual dyspraxia. First overnight-EEG, performed at 2 years 10 months of age, as part of formal work-up for the language delay showed during wakefulness a normal background with frequent runs of triphasic high-voltage spikes, occurring asynchronously in left fronto-centro-temporal and right fronto-temporal regions, with slightly right predominance. During sleep this activity became almost continuous, with a NREM-sleep SWI > 80% (Fig. 4). Valproic acid was started with some improvement of the attention disorder and no effect on language. At 3 years of age, he used about 3–5 referential two-syllable words with abundant dyslalia and mild oro-lingual dyspraxia. At the age of 3 years 2 months, he started to present sporadic and brief (up to 10 seconds) staring episodes without other associated signs. At this age a brain MRI was normal. Epilepsy was diagnosed at the age of 3 years 6 months after a seizure during sleep/wakefulness transition characterized by staring and bilateral "tremors" of the upper extremities. A neuropsychological evaluation at that time showed a global IQ of 46 (language 39, coordination 50, socialization 43). At 4 years of age a neuropsychological evaluation estimated a developmental age corresponding to 1 year. Since this age, despite the association of different treatments (clobazam, ethosuximide, sulthiame, zonisamide, rufinamide, lamotrigine, perampanel, lacosamide, corticosteroids and ketogenic diet) he continued to have weekly generalized tonic-clonic seizures, and hemiclonic seizures (on both sides) with or without loss of awareness, predominantly during sleep or awakening. Over the years, seizure frequency progressively decreased, and since the age of 7 years he is seizure free on valproic acid. Overnight EEGs continue to show a NREM SWI > 80%. At last follow-up at the age of 9 years, he presented motor aphasia, mild oro-lingual dyspraxia, and abnormal behaviour characterized mainly by impulsivity and irritability. He needed support to attend to a special school and for daily activities, and he continued speech therapy.

At 5 years of age, an array-CGH showed a deletion of 10 kb on Xp22.12, ChrX:21609392-21619786 involving the region of *CNKSR2* gene. Parents were tested and found negative.

4. Discussion

In this study we report the electro-clinical phenotypes of five novel patients with *CNKSR2*-related ESES, comparing them with

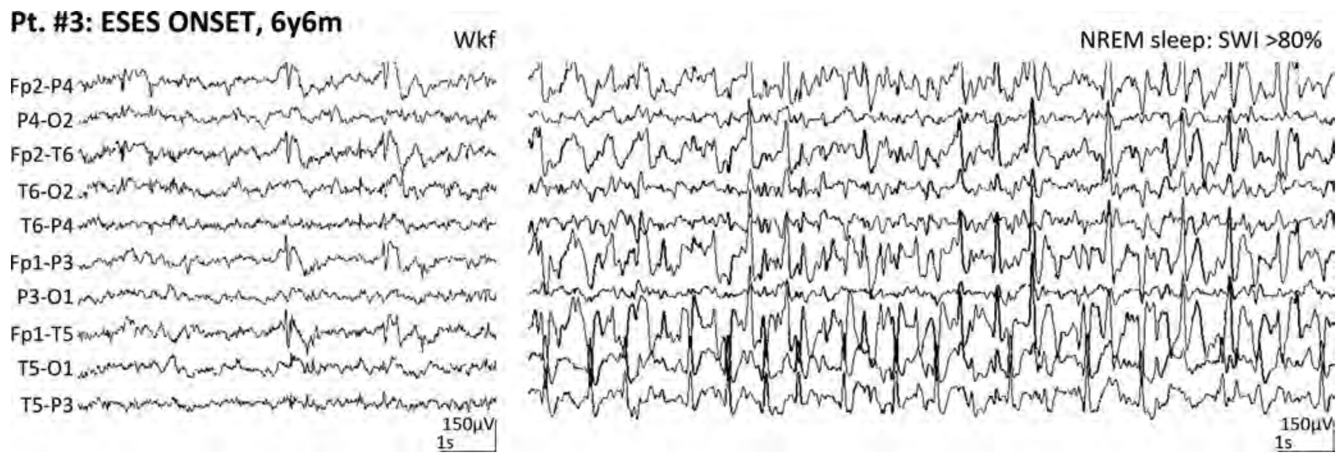

Fig. 3. Wakefulness and NREM sleep EEG recording in patient #3. At the age of 6 years 6 months, wakefulness (left panel) and NREM sleep EEG (right panel) in patient #3. During wakefulness (left panel), EEG disclosed multifocal spike-wave discharges. During NREM sleep (right panel), striking increment of bilateral fronto-temporal spike-wave discharges, with left predominance (NREM-SWI: >80%). Legend: EEG: electroencephalogram; ESES: encephalopathy related to status epilepticus during slow sleep; m: months; NREM: non-rapid eye movement sleep; Pt.: patient; y: years; Wkf: wakefulness.

previously published cases (Vaags et al., 2014; Damiano et al., 2017; Sun et al., 2018) (Table 1). Three of our patients (#1, #3, #5) presented with neurodevelopmental delay with severe language impairment since early infancy, as already described (Vaags et al., 2014; Damiano et al., 2017; Sun et al., 2018), whereas, the other two patients (#2, #4) developed normally until the onset of epilepsy at the age of 3 and 6 years respectively. Seizure onset was associated with the appearance (#2, #4) or aggravation (#1, #3, #5) of cognitive and/or behavioral disturbances. During the course of the disease, a further worsening of the cognitive and behavioral disorders was observed in coincidence with the detection of strikingly enhanced sleep-related EEG epileptiform discharges, leading to the diagnosis of ESES. In patients #2 and #4, long-term follow-up showed the remission of epilepsy and mild/-moderate cognitive impairment, indicating that *CNKSR2*-related ESES can present with a phenotype milder than previously reported in adult patients harboring *CNKSR2* pathogenic variants and presumed ESES in childhood (Vaags et al., 2014).

Bilateral diffuse, or unilateral, more or less focal (frontal, centro-temporal, parietal, occipital), subcontinuous epileptic discharges during sleep are the EEG features defining ESES (Tassinari et al., 2019). In 4/5 of our patients, topography of sleep-related EEG epileptic discharges during ESES was primarily frontal with spreading to central or temporal regions, in agreement with previous observations in patients with *CNKSR2*-related ESES (Table 1). This finding may suggest that the involvement of frontal areas by the epileptic activity, in particular during sleep, might be a distinctive EEG feature of *CNKSR2*-related ESES, at variance with ESES associated with *GRIN2A* pathogenic variants, in which the EEG focus is most often located more posteriorly, in centro-parietal regions (Gabrielle Rudolf, personal communication). Indeed, a recent paper has shown in two patients with *GRIN2A*-related ESES, a multifocal (parietal and temporo-parietal) topography of the EEG focus (Pavlidis et al., 2019). However, at present the data are very scanty to establish whether there are distinctive EEG patterns in the different genetically-determined ESES. The lack of a systematic neuropsychological assessment in our patients renders difficult to establish whether the type of cognitive/behavioral disorders may be related to the predominant frontal topography of sleep-related epileptic discharges or alternatively may depend on a more diffuse brain dysfunction, possibly related to impaired sleep homeostasis, as recently suggested (Pavlidis et al., 2019, Rubboli et al., 2019) as well as mediated by remote inhibition mechanisms (De Tiege et al., 2008).

All previously published patients harboring *CNKSR2* pathogenic variants are reported to suffer from language impairment, not further specified. Four out of five of our subjects presented with speech and/or oro-motor dyspraxia, unreported yet in *CNKSR2*-related disorders. Speech dyspraxia refers to impaired motor planning and programming whereas oro-motor dyspraxia impairs speech execution (Turner et al., 2015). As observed in *GRIN2A*-related EAS conditions (Turner et al., 2015), the combination of these speech dysfunctions could account for the severe language disturbance also in *CNKSR2*-related disorders. The observation of these speech disturbances in three patients (#1, #3, #5) before the diagnosis of ESES may suggest that speech/oro-motor apraxia may be a feature of *CNKSR2*-related encephalopathy rather than directly linked to ESES. These speech deficits, reported in all published patient with *CNKSR2* pathogenic variants, suggest that *CNKSR2* plays a role in speech production. In fact, *CNKSR2* has been shown to be expressed, since the prenatal age, in structures such as cerebellum and nucleus caudatus which are involved in speech production (Liegeois and Morgan, 2012).

Episodes of prolonged absence status epilepticus or aggravation of atypical absences have been observed in 3/5 patients (#1, #3, #4), in concomitance with the diagnosis of ESES. Based on previous data suggesting that worsening of epileptic seizures might herald the onset of ESES (Saltik et al., 2005), aggravation of epilepsy in patients harboring *CNKSR2* pathogenic variants should prompt clinicians to perform a sleep EEG to verify the occurrence of an ESES EEG pattern. Interestingly, in patient #1 aggravation of atypical absences was associated with a striking increment of EEG epileptic discharges during wakefulness (awake SWI: 90%), with persistence of continuous spike-wave activity in between the absence episodes. Further studies may clarify whether modifications/increase of awake SWI may correlate with epilepsy worsening during the course of ESES. It is noteworthy that the increment of epileptic EEG activity during wakefulness was not accompanied by further worsening of the cognitive and behavioral disturbances. This is in keeping with data suggesting that cognitive derangement in ESES might be linked to the impairment, caused by exaggerated EEG epileptic activity during NREM sleep, of physiological sleep-related cortical plasticity processes that underlies learning and memory consolidation, particularly in the developmental age (Tononi and Cirelli, 2014; Rubboli et al., 2019).

Herein we describe the first female (#4) with *CNKSR2*-related ESES. Previously three female patients with *CNKSR2* pathogenic variants were described (Damiano et al. 2017, Polla et al. 2019)

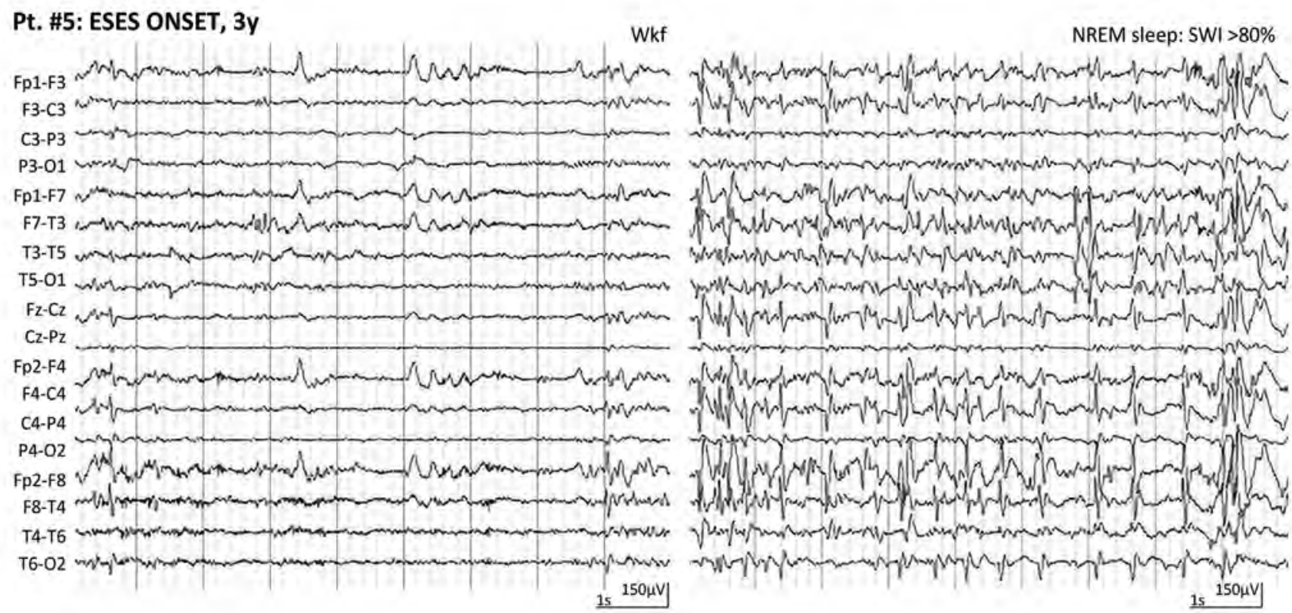

Fig. 4. Wakefulness and NREM sleep EEG recording in patient #5. At the age of 3 years, wakefulness EEG (left panel) showed high amplitude triphasic high-voltage spikes, in the right fronto-temporal regions and sporadic runs of delta activities in bifrontal regions were detectable. NREM sleep (right panel) was characterized by a remarkable enhancement of spike-wave activity (SWI > 80%), with right fronto-central/fronto-temporal predominance. Legend: EEG: electroencephalogram; ESES: encephalopathy related to status epilepticus during slow sleep; NREM: non-rapid eye movement sleep; Pt.: patient; y: years; Wkf: wakefulness.

associated with a mild phenotype of various different types of seizures with or without intellectual disability. One of them, sister of two brothers with *CNKSR2*-related ESES suffered from benign epilepsy with centro-temporal spikes, part of the spectrum of EAS (Damiano et al., 2017). Recently, an increasing number of X-linked conditions initially identified in affected males have also been reported in severely affected females (such us for instance *KIAA2022*, *IQSEC2*-related disorders, recently published by de Lange et al., 2016; Mignot et al., 2019), suggesting that additional mechanisms may contribute to the pathogenesis of these disorders other than those related to the classical heterozygous inheritance.

In conclusion, with this study, we expand the phenotypic spectrum of *CNKSR2*-related ESES including, as novel findings, occurrence also in females, normal neurodevelopment before epilepsy onset, mild/moderate intellectual disability in adulthood, epilepsy aggravation associated with enhanced awake SWI and speech/oromotor dyspraxia as the cause of language impairment. Additional studies may corroborate our findings helping clinicians in providing a correct and timely diagnosis.

Acknowledgments

We thank the patients and their parents for their kind cooperation.

Declaration of Competing Interest

None of the authors have potential conflicts of interest to be disclosed.

Funding

This work was supported by the Ministry of Science of Spain [project number SAF2014-59594-R] for JMS.

Author's contributions

CMB and GR reviewed the literature, performed clinical data collection and drafted the manuscript. CM, JMS, GL, BGG, RM, GR, KMJ, PMG contributed in collecting clinical data from patient's chart and from the literature. EG and CR revised and analyzed electrophysiological data. All authors revised the manuscript and approved the final article. GR, EG, RSM, CAT conceived the idea of the present paper, critically reviewed and finally approved the manuscript.

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