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A peculiar family with recurrent self-limited epileptic syndrome and associated developmental disorders in six girls

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ABSTRACT

We describe a complex family with two couples (two sisters who married two brothers) with consistent social and neuropsychiatric problems, originally from Sardinia. Each couple had three daughters, which shared electroclinical epileptic syndrome and developmental disorders. All patients suffered from mild to moderate intellectual disability, speech difficulties and behavioural disorders. Four out of six patients had epilepsy onset between 3 and 4 years of age. The epileptic history almost reflected the typical clinical course of a self-Limited Focal Epilepsy of Childhood. However, our patients don't have the complete features characteristic of one of the four specific self-Limited Focal Epilepsies of Childhood; a progressive evolution into a Developmental and/or Epileptic Encephalopathy with spike-wave activation in sleep was observed in the two older sister of the first family, which developed more severe developmental disorder too. In the other epileptic patients, improvement of EEG pattern was not coincident with an improvement of the developmental disorders. Brain MRI, performed in three patients, showed normal findings. Genetic analysis carried out so far (SNP-array, study of Runs of homozygosity, FMR1 tripletrepeat primer-PCR assay, Next Generation Sequencing based gene panel for epilepsy and neurodevelopmental disorders and Exome Sequencing), did not provide useful elements for an aetiological diagnosis. © 2022 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

According to the last proposed Position Paper by the ILAE Task Force on Nosology and Definitions, Self-Limited Focal Epilepsies of Childhood (SeLFE) syndromes are defined as focal epilepsies with childhood onset, usually of unknown cause [13].

Self-limited Epilepsy with Centrotemporal Spikes (SeLECTS) is the most frequent SeLFE and accounts for about 6–7% of all childhood epilepsies [17,18].

Development, cognition, neurological examination and head size prior to seizure onset are typically normal. While the epilepsy is active, behavioural and neuropsychological deficits may emerge or worsen, particularly in language and executive functioning [8,9]. Some patients present with features of autistic spectrum disorder, but, unlike primary autism, there is no loss of social interaction [5]. These deficits often improve or resolve with age [4].

Some individuals with SeLECTS may show evolution to a Developmental and/or Epileptic Encephalopathy with spike-wave activation in sleep (D/EE-SWAS), with associated language and cognitive impairment, a broad spectrum of syndromes with various combination of clinical and EEG features [6].

Genetic factors play an important etiological role, as supported by the higher incidence of a positive family history for epilepsy or febrile seizures, and age-dependent, focal EEG abnormalities in the patients' relatives. However, it is well known that the genetic component of SeLECTS is consistent with complex inheritance patterns [16]. We describe the electroclinical phenotype of a Sardinian family with six affected girls and history of epilepsy, developmental disorders and psychiatric diseases in their parents.

2. Case report

We described the electroclinical and genetic features of two Sardinian families. Two sisters married two brothers and both couples had three daughters, currently aged between 5 and 14 years.





Fig. 1. Genealogical three. (I:2; psychotic disorder/trouble with the law, I:4; Major Depressive Disorder).



Fig. 2. Patient II:1, Initial EEG recording at seizure onset showing fronto-centro-temporal spikes with activation during sleep (2010).

Both parents' families originated from a south-west Sardinian area, called "Sulcis". No prior consanguinity between the two families was reported. Miscarriage was reported in the first couple but the sex of the fetus remained unknown. The two fathers suffered from mood and behavioral disorder; in particular, one of them suffered from a psychotic disorder and had trouble with the law (Fig. 1, I:2). The other had major depressive disorder (Fig. 1, I:4). Both were not treated with antipsychotic drugs. The mothers, both presented with mild intellectual disability, and one of them (Fig. 1, I:1) also had epilepsy (not well characterized) in childhood; she died because of traumatic causes. All the daughters had normal pregnancy history and normal motor milestones acquisition prior seizure onset except for expressive language delay. No dysmorphic features were observed in any of them.

Four children (Fig. 1, patient II:1, II:2, II:4 and II:6) had epilepsy onset between 3 and 4 years of age. All of them presented with focal motor seizures during sleep (clonic contraction of eyes and mouth, sialorrhea). EEG recordings showed, at epilepsy onset, asynchronous bilateral high amplitude centrotemporal sharpand-slow wave complexes, showing alternating side prevalence, activated during drowsiness and sleep, with normal background activity and normal sleep architecture (Fig. 2). The same EEG pattern was found in patient II:3 too (Fig. 1), although she never presented epileptic seizures. The EEG recording performed on the patient II:5 (Fig. 1) was normal.

Two sisters of the first family (Fig. 1, patient II:1 and II:2) had a progressive worsening of the eletroclinical pattern with an evolution into a D/EE-SWAS [13]. During school age they developed a continuous spike-and-slow wave/polyspike-and-slow wave EEG pattern in sleep (SWAS) (Fig. 3). This EEG pattern was associated with frequent seizures during sleep and with worsening of their neuropsychiatric problems. To achieve complete seizure control, three antiseizure drugs were needed (valproic acid, clobazam and ethosuximide).



Fig. 3. Patient II:1, EEG recording showing electroclinical worsening, with bursts of spike-and-waves most evident in fronto-central regions and a Spike Index > 90%. (2013).

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Fig. 4. Patient II:1, EEG recording after epilepsy resolution showed a normal pattern (2018-2020).

Patients II:1 and II:2 showed subsequent electroclinical improvement (Fig. 4) with seizure interruption and progressive normalization of the EEG pattern occurred between 8 and 12 years of age, and plateauing of behavioural disorders. Patient II:4 (Fig. 1) showed good response to drug monotherapy with Levetiracetam and an electroclinical improvement from 8 years of age, with complete epilepsy resolution; however, there was no subsequent improvement in behavioural aspects. Patient II:6 (Fig. 1) had epilepsy onset at 4 years of age, no worsening of the electroclinical pattern, that remains stable until now, and showed a good response to pharmacolog-

ical treatment with low seizure rates despite persistent neuropsychological deficits.

All patients suffered from mild to moderate intellectual disability and speech disorders of varying degrees. Patient II:1 and II:2, who had the worst electroclinical pattern, developed a more severe developmental disorder too, if compared with the others; the oldest also had severe deficit in social communication and interaction (not formally diagnosed as Autism Spectrum Disorder) and behavioral disorders. Speech disturbance observed in the patients may be due to isolated delayed speech prior to seizure onset, and subsequent plateauing of language acquisitions due to neuropsychiI. Cursio, N. Ronzano, M. Asunis et al.

VPA: valproic acid.	ETS: ethosuximide. CLB: clobazam. LEV	: levetiracetam.				
Patient	II:1	II:2	II:3	II:4	II:5	II:6
Seizure onset	3 years of age	3 years of age	No seizures	3 years of age	No seizures	4 years of age
EEG	Typical SelECTS EEG evolved into D/EE-SWAS. From 12 years of age, improvement	Typical SeLECTS EEG evolved into D/ EE-SWAS. From 8 years of age, improvement of	Typical SeLECTS EEG	Typical SeLECTS EEG- From 8 years of age, improvement of	Normal	Typical SeLECTS EEG
Developmental disorders	or erectroctinucal parterin. Moderate intellectual disability, speech and behavioral disorder, relational difficulties	etectrochinical patterin. Mild intellectual disability, speech and behavioural disorder, improved from 8 vears of age	Mild intellectual disability and learning disorder	electrochineal partern Moderate intellectual disability and speech disorder	Mild intellectual disability, speech and learning disorder	Mild intellectual disability and speech disorder.
Seizure' semiology	Focal motor during sleep, then focal non motor during sleep	Focal motor during sleep		Focal motor during sleep		Focal motor during sleep
Brain MRI Neurological	Normal Mild kinetic tremor affecting upper	Normal Mild kinetic tremor affecting upper	Mild kinetic tremor	Mild kinetic tremor affecting	Mild kinetic tremor	Normal Mild kinetic tremor
examination	extremities, coordination deficit	extremities, coordination deficit	affecting upper extremities, coordination deficit	upper extremities, coordination deficit	affecting upper extremities, coordination deficit	affecting upper extremities, coordination deficit
ASMs	VPA, ETS, CLB.	VPA, ETS, CLB		LEV		LEV/ETS

atric problems. In both parents' couple wasn't reported any language disturbance, neither was observed any speech dysfunction. At neurological examination, mild kinetic tremor affecting upper extremities was found in all patients, associated with coordination deficit (Table 1); we could explain it as a coincidental factor, rather than an actual movement disorder.

Clinical presentation of both psychiatric disorders of the fathers and the neuropsychological deficit in the mothers were more severe in the couple with the daughters who had the worst elettroclinical presentation.

Only patients II:1, II:2, II:3 and II:4 (Fig. 1) received a formal cognitive assessment using Wechsler scales. Because of children's behavioural problems and familial background with consistent social problems, the cognitive and global assessment was very difficult to perform. Sometimes, it remained unfinished and not estimable with batteries of structured tests, because of the parents' lack of cooperation at standardized evaluation of their daughters.

Patient II:1 (Fig. 1) received cognitive assessment using Wechsler Intelligence Scale for Children (WISC- IV) scale either during the electroclinical worsening and then at the resolution of the SWAS pattern (in 2018), showing global lower index scores in the first evaluation, and test subministration was difficult, due to behavioural problems. A global heterogeneity among scores was reported, showing working memory and processing speed index scores were lower than those for verbal comprehension and perceptual reasoning. Patient II:2 (Fig. 1), was assessed using WISC-IV and had an IQ of 59. Working memory (score: 55) and processing speed index (score: 56) scores were lower than those for verbal comprehension (score: 84) and perceptual reasoning (score: 74). Patient II:3 (Fig. 1) was assessed using Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III) (2019), showing the following index score: Full scale IQ: 73, Verbal IQ:78, Performance IQ: 73, processing speed:74. Patient II:4 (Fig. 1) received cognitive assessment using WISC- IV in 2019, that showed IQs: 47, with the following index scores: verbal comprehension (score: 68) and perceptual reasoning (score: 56), working memory (score: 61) and processing speed (score: 53).

Brain MRI was performed in three patients (Fig. 1, patient II:1, II:2 and II:6), those with the worst clinical phenotype and the youngest one, and showed normal findings.

3. Genetic analysis

We performed genetic analysis in all the daughters (Table 2). The children underwent tests of single nucleotide polymorphism (SNP) array for chromosomal abnormalities, using the Infinium Cytosnp 850 K v1.2 microarray, spanning the entire genome, with a resolution of 100 Kb, according to the instructions provided by the manufacturer. No pathogenic copy number variants (CNVs) were found.

The study of runs of homozygosity (ROH) revealed one DNA region identical in all the patients studied. This region, extending 4732 Kb, located at chromosome Xp22.2 is (10360284_10833480), and includes a single gene: MID1. Pathogenic variants in the MID1 gene are responsible for the X-linked form of Opitz syndrome (XLOS), a multiple congenital disease characterised by defects in the development of midline structures during embryogenesis. The most frequently observed signs are: dysmorphic features (hypertelorism, often associated with cleft of lip and palate, frontal bossing, large nasal bridge, low-set ears, etc.), laryngo-tracheoesophageal abnormalities, external genitalia abnormalities, but also cardiac abnormalities, anal defects, as well as other less frequent signs. XLOS also presents a neurological component presented as anatomical cerebellar vermis hypoplasia and agenesis or hypoplasia of the corpus callosum as well as cognitive

Patient	II:1	II:2	II:3	II:4	II:5	II:6
SNP - array ROH	No CNVs Xn222 (10360284 10833480)	No CNVs Xn22 2 (10360284 10833480)				
	473,2 Kb	473,2 Kb	473,2 Kb	473,2 Kb	473,2 Kb	473,2 Kb
FMR1 CGG	20/30	20/30	20/30	30/30	30/30	20/30
repeats						
NGS panel	No pathogenic variants	No pathogenic variants	No pathogenic variants	No pathogenic variants	No pathogenic variants	No pathogenic variants
ES	No pathogenic variants	No pathogenic variants	No pathogenic variants	No pathogenic variants	No pathogenic variants	No pathogenic variants

and developmental delays [1]. However, the XLOS phenotype does not correspond to our patients' features.

In addition, in order to rule out a Fragile-X syndrome, FMR1 triplet-repeat primer (TP)-PCR assay (AmplideX PCR/CE FMR1 Kit; Asuragen) was performed, which revealed a normal genotype in all patients [2,10].

A targeted gene panel including 370 genes related to epilepsy and neurodevelopmental disorders was performed in Patients II:1 and II:4 without identifying any causative variant. Subsequently, an exome sequencing (ES) was carried out in all the available individuals. Since none of the parents was available. ES analysis was performed only in the siblings without applying any transmission model. In brief, ES variants were annotated with the VarSeq software (Golden Helix, USA) and retained if absent or with a low frequency (<0.1%) in the gnomAD population database. Analysis was focused on the variants resulted to be shared by at least four out of six patients, independently from zygosity (heterozygous or homozygous), and excluding patients without a variant genotype (reference) or missing (not genoyped). None of the selected variants was deemed as causative in the patients.

4. Discussion

All patients showed an almost identical association between EEG patterns and developmental disorders (intellectual disability, language impairment, mild movement disorder) at various degrees of severity. The epileptic history of our patients, in terms of age of onset, EEG pattern, seizure semiology and EEG normalization with seizure resolution at 8-12 years of age reflected the typical clinical course of a self-limited focal epilepsy of childhood. However, our patients don't have the complete features characteristic of one of the four specific self-Limited Focal Epilepsies of Childhood [15], neither they had a mixed picture. Because of EEG pattern and seizure semeiology, self-limited epilepsy with centrotemporal spikes is the most comparable to our patients presentation. A specific characteristic of this syndrome is good response to antiseizures medications and simultaneous improving or resolution of behavioural and neuropsychological deficits [3]. Among the daughters of the two families described, instead, two sisters (Fig. 1, patient II:1 and II:2) showed electroclinical pattern evolving in a D/EE-SWAS, in which the epileptiform abnormalities themselves contributed to the progressive disturbance in cerebral function, and the patients developed a drug resistant epilepsy and a global regression of cognitive function [3]. The resolution of electroclinical pattern was not coincident with an improvement of the developmental disorders. In addition, we observed different outcomes among the four daughters who developed epilepsy. We think that this could be the result of genetic susceptibility, EEG features and evolution, seizure frequency, and that the electroclinical course of each patient was almost independent from antiseizure medications.

Because of the family history of behavioral and developmental disorders among the parents' couples and the shared clinical and instrumental characteristics among the daughters, we obviously firstly supposed a common genetic background in this family. In particular, we supposed a causative role of GRIN2A gene, that recently emerged as the major candidate for the SeLFE syndromes with an uncommon presentation and a variable prognosis [11,12,14]. However, the lack of significant results in the genetic analysis by NGS did not confirm our hypothesis. ES analysis didn't provide any additional information. Moreover, SNP array analysis revealed an only run of homozygosity identical in all the the six daughters at chromosome Xp22.2. Unfortunately it seems that this DNA region doesn't include any gene so far described as associated with the patients' clinical phenotype. Additional sequencing stud-

ies will be carried out in order to look for variants in this DNA region not covered by WES, possibly extending our study to available parents in the two families.

5. Conclusions

Wide clinical variability within the spectrum of SeLFEs underlies a broad convergence of genetic factors, with different models of inheritance. This atypical presentation and evolution in the patients of our families and the association between the described electroclinical and developmental features has not yet been defined in literature. Il would be important the early recognition of this electroclinical phenotype because of its impact on development, despite a globally good epileptic prognosis. This opens the search for other families with similar characteristics. Therefore, it would be advantageous to identify genetic risk factors at an early stage in the clinical course and to study family cases within populations with a complex genetic background, such as the Sardinian population [7].

Ethical statement

Informed consent was obtained from individuals' tutors discussed in this publication.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

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