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Case Report

Expanding the electro-clinical phenotype of *CARS2*associated neuroregression

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ABSTRACT

Biallelic variants in *CARS2* (Cysteinyl-tRNA synthetase 2; MIM*612800), are known to cause combined oxidative phosphorylation deficiency 27 (MIM#616672), characterized by severe myoclonic epilepsy, neuroregression and complex movement disorders. To date, six individuals from five families have been reported with variants in *CARS2*. Herein, we present an 11-year-old boy who presented with neuroregression, dysfluent speech, aggressive behavior and tremors for 2 years. An electroencephalogram (EEG) revealed a highly abnormal background with generalized spike-and-wave discharges suggestive of Electrical Status Epilepticus during Sleep (ESES). A known homozygous c.655G > A(p.Ala219Thr) pathogenic variant in exon 6 of the *CARS2*(NM_024537.4) was identified on exome sequencing. Our report expands the electro-clinical spectrum of the phenotype with presence of severe behavioral abnormalities, continuous tremors and ESES pattern on EEG, not previously reported.

Introduction

The clinical findings in mitochondrial disease tend to be heterogeneous, but central nervous system involvement is common. Mitochondrial disease is often suspected in children presenting with an epileptic encephalopathy, cognitive decline, complex movement disorder and non-specific findings on neuroimaging. Pathogenic variants in cysteinyl-tRNA synthetase2 (*CARS2*) gene have been recently described to be associated with severe myoclonic epilepsy, neuroregression, progressive tetraparesis, progressive visual and hearing impairment and complex movement disorders including chorea, dystonia, oculogyric episodes, myoclonus and startle myoclonus [1–4]. Neuroimaging of the brain in these individuals has revealed abnormalities such as white matter abnormalities, a thin corpus callosum, cerebral atrophy and cerebellar hypoplasia [1,3–5]. We report a 11-year-old boy with a known homozygous pathogenic variant c.655G > A in *CARS2* presenting with neuroregression, dysfluency in speech, aggressive behavior and tremors and ESES pattern on EEG over 2 years expanding the phenotypic spectrum of *CARS2*-related disorder.

Clinical report

A 11-year-old male child, second born to a second degree consanguineous couple, presented with progressively worsening behavioral issues, speech dysfluency, memory deficit and cognitive decline for the past 2 years. He was born by normal delivery at term with birth weight of 2.6 kg (normal). His perinatal history was unremarkable. He was developmentally normal till about 9 years of age when the parents started observing changes in behavior in the form of aggression and poor temper control. They also noticed change in speech in form of dysfluency and unclear content. He was also observed to develop deficits in memory and progressively worsening scholastic performance leading to discontinuation of schooling after 5th grade. The parents also noticed onset of continuous tremors of both the hands, left more than right, which used to subside only in deep sleep. They were involuntary, rhythmic to and fro movements of hands. There was no history of seizures including no myoclonus and no drop attacks. There was no significant family history of people with similar features.

On examination, his height was 135 cm (−1.5 SD), weight was 29.5 kg (−0.99 SD), BMI 16.19 (−0.52 SD). Clinical examination revealed intact comprehension with poor speech output. The speech was barely understandable with marked dysfluency [1–8]. The presence of continuous tremors involving both the hands was observed. Neurological examination revealed normal power in all four limbs. Deep tendon reflexes were elicitable and were

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not brisk. Bilateral plantar reflexes yielded a flexor response. Vision and hearing were normal. No obvious dysmorphism or neurocutaneous markers were observed. His systemic examination was normal.

Clinical investigations including thyroid function tests were normal. Blood gas analysis, serum lactate level sand liver function tests were within normal limits. Ultrasound abdomen revealed normal size and architecture of the liver. The metabolic parameters including amino acid and acyl-carnitine levels were within normal limits. Brainstem auditory evoked responses (BAER) suggested normal hearing in both the ears. MRI of brain revealed no significant pathology. The patient was sedated using Triclofos. The 30-minute continuous sleep electroencephalogram (EEG) using the International 10–20 system of electrode placement, 22 electrodes, and a bipolar longitudinal montage revealed a highly abnormal background with no sleep markers and generalized spike-and-wave discharges suggestive of electrical status epilepticus during sleep (ESES) (Fig. 1).

Genetic testing

Informed consents approved by the institutional ethics committee was obtained from the family. Genomic DNA was isolated from the EDTA blood samples of the proband and his parents. A known homozygous pathogenic variantc.655G > A (p.Ala219Thr) in exon 6 of the *CARS2* (NM_024537.4) was identified in the proband in concordance with the observed phenotype [1]. Sanger validation and segregation analysis showed the presence of this variant in the homozygous state in the proband and the heterozygous state in the parents.

Discussion

CARS2, first identified by Bonnefond et al [2], is a nuclear encoded 564-amino acid protein involved in mitochondrial translation. This gene encodes a putative member of the class I family of aminoacyl-tRNA synthetases. These enzymes play a critical role in protein biosynthesis by charging tRNAs with their cognate amino acids. Hallmann et al [1] was the first group to establish a disease-gene association of *CARS2* with neurodegenerative disorders and progressive myoclonic epilepsy.

Only six cases of *CARS2* variants have been described in literature to date, including three children and three adults [1,3–6]. All the reported cases had developmental delay or neuroregression with cognitive decline and drug-resistant epilepsy. Vision and hearing impairment were present in two patients and complex movement disorders were observed in two patients. Table 1 compares the genotype and clinical symptoms of our study subject with the six patients previously reported, including two siblings from the same family (Patient 1 and 2).

There were some unique observations in our patient. The clinical presentation in our case was characterised by the presence of severe behavioral abnormalities in the form of aggression, destructive behavior, violence and use of abusive language, along with continuous tremors of both hands. The most common seizure semiology described in association with CARS2 include generalisized tonic-clonic seizures, focal seizures, and myoclonic seizures [1,3,4]. Though there was no history of clinical seizures in our case, his sleep EEG revealed highly abnormal background with generalized spike-and-wave discharges suggestive of ESES. Since ESES has been associated with cognitive decline and behavioral abnormalities in children [7], this could explain the behavioral symptoms in our case, even in absence of clinical seizures. This EEG pattern has not been reported previously in contrast to reported EEG observations including generalized spike-wave discharges, multifocal epileptiform discharges, and focal occipital and frontal epileptiform discharges [1,3–6]. The unique behavioral manifestations and EEG findings in our patient adds to the electro-clinical spectrum of this condition.

We considered Landau Kleffner Syndrome (LKS) a close differential in our case due to presence of behavioral abnormalities and ESES pattern on EEG. However, the age of onset of symptoms and lack of verbal auditory agnosia and aphasia made LKS a less likely possibility in our case. Genetic testing further confirmed the presence of a pathogenic variant in the *CARS 2* gene.

Disease-causing variants in *CARS2* have been identified in a total of six individuals from five families with six missense variants and one in-frame deletion (Table 1) [1,3–6]. The missense variant c.655G > A identified in the individual in our study has been previously reported in two siblings from a consanguineous family [1]. The variant has been classified as 'pathogenic' using the American College of Medical Genetics and Genomics (ACMG) sequence variants interpretation guidelines criteria PVS1, PS3, PM2, and PP5 [8].

Management of this disorder remains supportive. Our patient received methylprednisolone pulse therapy (30 mg/kg/day) for 3 days followed by oral prednisolone (2 mg/kg/day) for 4 weeks, followed by tapering doses along with monthly doses of intravenous immunoglobulin (1 g/kg). He has also been receiving antiseizure medication (levetiracetam and clobazam) over the last 6 months but has shown minimal clinical and electrophysiological improvement (EEGs were repeated after 4 weeks and 12 weeks after starting therapy) during follow-up visits. Though there was mild improvement in his aggressive behavior, the tremors and abnormal EEG findings persisted.

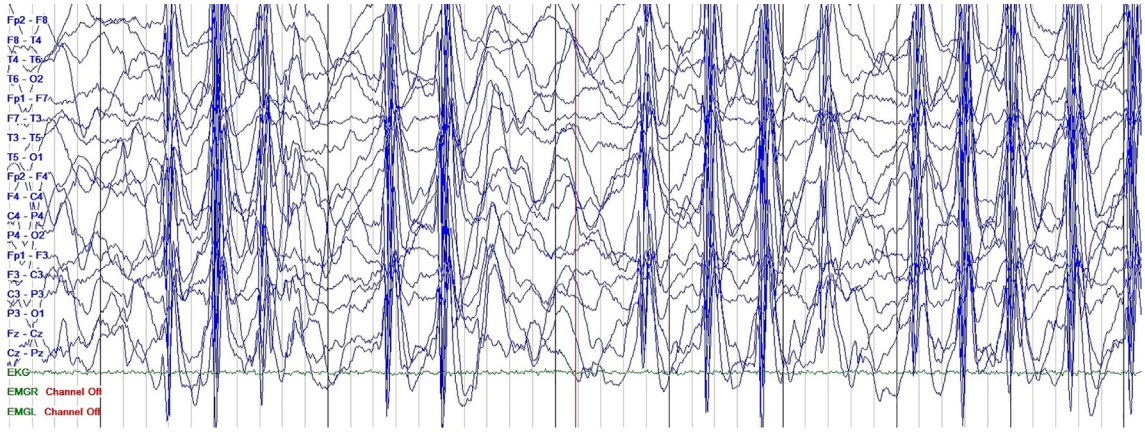

Fig. 1. EEG showing continuous spike and wave discharges during sleep suggestive of Electrical status epilepticus during sleep (ESES) (Longitudinal bipolar montage, Sensitivity 70uV/cm, Low Frequency Filter: 1 Hz, High Frequency Filter: 70 Hz).

Table 1
The mutations and clinical features of previously reported cases and our study subject.

Clinical features	Present study	Hallmann et al., 2014		Coughlin et al., 2015	Samanta et al., 2018	Hu et al., 2020	Wu, Teng Hui et al., 2020
		1-IV-1	2-IV-2				
Age at last examination	11 years	28 years	18 years	3 year 10 months	13 year	-	4 years 7 months
Age at onset	9 years	9 years	5 years	5 months	12 year	6 months	-
Gender	Male	Male	Female	Male	Female	Male	-
Variants in *CARS2*				(NM_024537.4) c.649_651delGAG(p.Glu217del); c.752C > T(p.Pro251Leu)	c.655G > A (p.Ala219Thr) c.155 T > G (p.Val52Gly); c.563C > T (p.Thr188Met)	c.655G > A (p.Ala219Thr) c.1426G > Tp.(Gly476Arg)	c.655G > A (p.Ala219Thr) c.1036C > Tp.(Arg346Trp); c.323 T > Gp.(Phe108Cys)
Zygosity	Homozygous	Homozygous	Homozygous	Compound heterozygous	Compound heterozygous	Homozygous	Compound heterozygous
Clinical features	Neuroregression, dysfluency in speech, aggressive behavior and tremors	Severe myoclonic epilepsy, progressive tetraparesis, visual and hearing impairment, and progressive cognitive decline	Severe myoclonic epilepsy, progressive tetraparesis, visual and hearing impairment, and progressive cognitive decline	Episodes of opisthotonus, feeding difficulties, developmental delay, refractory seizures, complex movement disorder with chorea, dystonia	Epilepsy, intellectual impairment, dysphagia, autism spectrum disorder, history of generalized pain and upper extremity tremors, focal status epilepticus.	Neuroregression, refractory seizures	Seizures, abnormal behavior, no eye contact, intermittent excitement, stereotype language and actions.
Investigations	Normal liver function tests and serum lactate level	Normal lactate level	Slightly raised lactate level	Normal liver function tests, raised lactate level	Elevated liver enzymes and lactate level	Normal CPK and lactate level	Not available
EEG	Abnormal background with generalized spike-and-wave discharges suggestive of ESES.	Generalized grouped spike and double spike wave complexes	Generalized grouped spike and double spike wave complexes	Multifocal epileptiform discharges	Left occipital pseudoperiodic epileptiform discharges	Not available	Bilateral frontal poles, temporal spikes, sharp waves, and sharp slow waves.
Neuroimaging	Normal	Bilateral white matter lesions in the occipital lobe and the cerebellum as well as global brain atrophy	Subtle white matter lesions in the left parietal lobe and brainstem	Atrophy of the cortex and, with focal increased T2 signal in cortex and white matter, a very thin corpus callosum and atrophic cerebellar vermis	Global cerebral and cerebellar atrophy, thinning of corpus callosum	Minor abnormality	Brain atrophy

Our observations are similar to those of Samanta et al [4] who tried intravenous immunoglobulin, high dose methylprednisolone, and a mitochondrial cocktail in their patient but did not observe any significant improvement in symptoms. The role of mitochondrial cocktails in this condition merits further research.

Our report aims to broaden the electro-clinical spectrum of *CARS2* associated neuroregression to further improve our understanding about this rare disorder. We also intend to highlight that clinicians should suspect and judiciously plan genetic testing methodology (like NGS genetic testing including whole genomic sequencing panel) for genetic diagnosis in children presenting with neuroregression, drug-resistant epilepsy, behavioral problems, movement disorders, abnormal epileptiform EEG patterns even in the absence of clinical seizures, when normal or non-contributory findings are pesent on metabolic evaluations and neuroimaging.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DK wrote the initial manuscript, AA helped in collecting data, SS modified the manuscript, PM and AS conducted the Genetic test and provided the inputs for modification of the manuscript, All the authors read and approved the final manuscript. We also want to thank our EEG lab technicians Mr Manas, Mr Kishan and Mr Kishan for their contribution.

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