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Case study

Abnormal circadian rhythm in patients with *GRIN1*-related developmental epileptic encephalopathy

Marcello Scala [a,b,*], *Elisabetta Amadori* [c], *Lucia Fusco* [d], *Francesca Marchese* [c], *Valeria Capra* [b], *Carlo Minetti* [a,c], *Maria Stella Vari* [c], *Pasquale Striano* [a,c]

[a] *Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy*
[b] *Department of Neurosurgery, IRCCS Istituto Giannina Gaslini, Genoa, Italy*
[c] *Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy*
[d] *Department of Neuroscience, Bambino Gesù Children's Hospital, Rome, Italy*

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ABSTRACT

GRIN1 encodes the obligate subunit (GluN1) of glutamate N-methyl-D-aspartate receptor (NMDAr). Pathogenic variants in *GRIN1* are a well-known cause of infantile encephalopathy characterized by profound developmental delay (DD), variable epileptic phenotypes, and distinctive behavioral abnormalities. Recently, *GRIN1* has also been implicated in the pathogenesis of polymicrogyria (PMG).

We investigated two patients presenting with severe intellectual disability (ID), epilepsy, stereotyped movements, and abnormal ocular movements. They showed distinctive circadian rhythm alterations and sleep-wake patterns anomalies characterized by recurrent cyclic crying or laughing spells. Genetic analysis led to the identification of two distinct *de novo* variants in *GRIN1* affecting the same amino acid residue of an important functional protein domain.

Recent advances in circadian rhythm and sleep regulation suggest that abnormal GluN1 function might play a relevant pathogenetic role for the peculiar behavioral abnormalities observed in *GRIN1* patients. Our cases highlight the relevance of circadian rhythm abnormalities in epileptic children as a clue toward *GRIN1* encephalopathy and expand the complex phenotypic spectrum of this severe genetic disorder.

* *Corresponding author*. IRCCS Istituto Giannina Gaslini, Via Gerolamo Gaslini 5, 16147, Genoa, Italy.
E-mail address: marcelloscala87@gmail.com (M. Scala).

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1. Introduction

Glutamate is the predominant excitatory neurotransmitter throughout the central nervous system (CNS), exerting its excitatory effect through ionotropic and metabotropic receptors. The ionotropic N-methyl-D-aspartate receptor (NMDAr) is a tetrameric voltage-dependent and ligand-gated ion channel permeable to sodium, potassium, and calcium.[1] *GRIN1* encodes the two obligatory glycine-binding GluN1 subunits of NMDAr, which further comprises two variable glutamate-binding GluN2 subunits (encoded by *GRIN2A-B-C-D*), a GluN2 subunit associated with a glycine-binding GluN3 subunit (encoded by *GRIN3A-B*), or two GluN3 subunit.[1] *GRIN1* pathogenic variants are responsible of an early-onset epileptic encephalopathy characterized by distinctive electro-clinical features, including heterogeneous seizure manifestations, intellectual disability (ID)/developmental delay (DD), stereotyped movements, and abnormal ocular movements.[2] We report two patients carrying *de novo* heterozygous *GRIN1* variants and showing rare and distinctive circadian rhythm alterations, further delineating the complex phenotypic spectrum of this disorder.

2. Case studies

2.1. Patient 1

This is a 17-month-old boy with history of infantile spasms and cyclic crying spells. He was the second born to unrelated healthy parents and his sister was healthy. The baby was delivered at term after uncomplicated pregnancy. Neonatal course was uneventful and his growth and development were normal in the first few months of life. At the age of 4 months, he showed significant irritability after the administration of the second dose of anti-meningococcal vaccination. He also started to adduct his arms to the chest, tightly clenching the fists. Two months later, after the third dose of vaccine, the baby developed West syndrome with cluster of epileptic spasms. Neurologic examination revealed psychomotor regression and dyskinetic movements of upper limbs. Electroencephalogram (EEG) showed hypsarrhythmia. Brain magnetic resonance imaging (MRI) was normal. Metabolic tests (acylcarnitine profile, cerebrospinal fluid analysis, neurotransmitters profile) yielded normal results. The patient was then started on adrenocorticotropic hormone (ACTH). Despite the good seizure control, he suddenly developed inconsolable though self-resolving crying spells, cyclically recurring every 10 days and apparently improving with clonazepam. These episodes were characterized by stereotyped mournful crying without tears, preceded by irritability and sleep discontinuity. Each spell lasted for an average of 5 days, during which the child experienced relevant circadian rhythms alterations. Sleep deprivation was significant. After 4–5 h of persistent crying, he was able to nap for just 15–20 min before waking up again. Feeding was almost impossible due to severe impairment of suction and swallowing during the crying spell. He also had lateral head nodding and flexion-extension movements involving both upper and lower limbs (See supplementary video material, Video 1 and Video 2, respectively). His EEG did not show epileptic activity. These symptoms were partially relieved by diazepam (2 mg/kg/day). The cause of these crying spells remained unknown despite thorough clinico-radiological evaluations. Analgesics and antacids were tried in suspicion of a possible ache or discomfort due to gastroesophageal reflux. Chronic constipation was treated with polyethylene glycol laxatives. However, no significant clinical improvement was noticed. Trazodone, promazine, and mirtazapine resulted ineffective for sleep-wake anomalies, whereas sleep duration partially improved with niaprazine. Dyskinetic movements responded to trihexyphenidyl hydrochloride.

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At the age of 17 months, neurologic examination revealed impaired visual fixation with afinalistic upward and lateral eye movements, severe diffuse hypotonia with lack of head control, hyperreflexia, and diffuse dyskinetic movements involving head, orofacial region, trunk, and limbs. The patient was nonverbal. Mild dysmorphic features included long face, high anterior hair line, short philtrum, hypertrophic gums, high arched palate, retrognathia, and large ears (Fig. 1A).

Video-EEG monitoring and visual and brainstem auditory evoked potentials were unremarkable. Targeted re-sequencing for epileptic encephalopathies led to the identification of the pathogenic variant c.2443G > A,p.(Gly815Arg) in *GRIN1*. Parental testing revealed that this variant was *de novo*.

2.2. Patient 2

This is a 15-year-old female referred for severe intellectual disability, spastic tetraparesis, and refractory epilepsy. She was the second-born to unrelated healthy parents and her sister was healthy. She was delivered at the 36th weeks' gestation after an uncomplicated pregnancy. Physical examination at birth showed severe diffuse hypotonia. The baby experienced several self-limiting apneic episodes in the first day of life. Neonatal course was further complicated by failure to thrive due to poor suction. DD was noticed in the first few months. The baby lacked head control, and showed dystonic and choreiform movements of upper limbs which partially responded to levodopa. At the age of 12 months, she started to suffer from right tonic seizures with head and gaze deviation. Seizures occurred frequently throughout the day, both in wake and sleep, and were associated with hyperhidrosis, pallor, perioral cyanosis, and tachycardia. She also developed stereotyped movements resembling hand-washing. Treatment with carbamazepine (20 mg/kg/day), vigabatrin (60 mg/kg/day), and zonisamide (10 mg/kg/day) were tried, alone or in combination, but resulted ineffective. Partial seizure control was achieved with valproate (30 mg/kg/day). By the age of 3 years, monthly cyclic episodes of unmotivated laughing and crying were noticed. During each episode, three days of recurrent laughing spells with significant feeding difficulties preceded three days of prolonged sleep, and further three days of crying spells. Acetaminophen

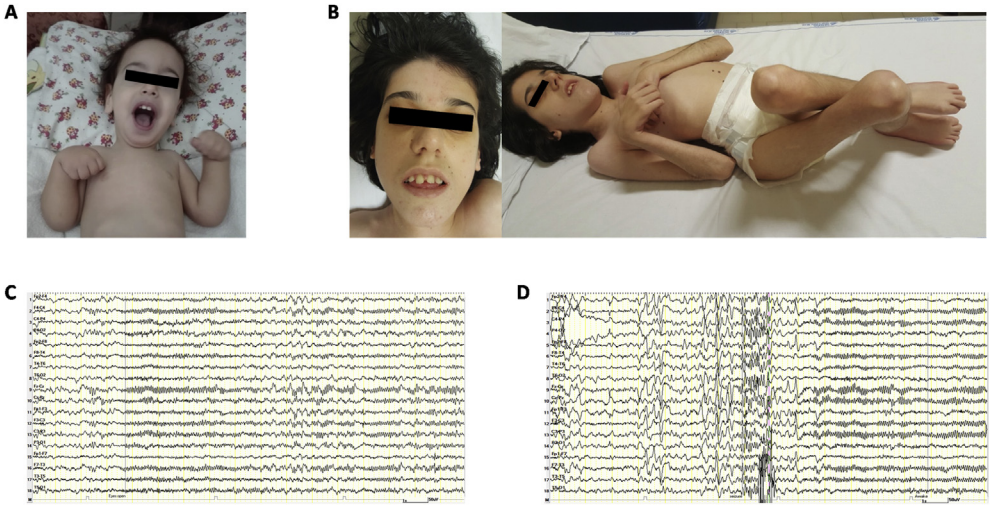

Fig. 1 – Electro-clinical findings. Clinical appearance of patient #1 (A) at the age of 17 months showing facial dysmorphic features, i.e., left eye esophoria, long face, high anterior hairline, large ears, and clenched hands; patient #2 (B) at the age of 15 years shows long face, long philtrum, widely spaced large teeth, severe scoliosis, decreased muscle mass, flexion contractures, and dystonic (distal) posture. EEG recordings (system 10–20, bipolar longitudinal montage, sens 10 μV, HF 50 RP Hz, LF 0.1, 20 sec/page) of patient #2 at age 15 years reveals rhythmic spindle activity (13–14 Hz) over the vertex and frontocentral regions at eye opening (C) and ictal burst of diffuse high-amplitude sharp waves associated with limb hypertonia, right head rotation and impaired contact (D).

and melatonin were ineffective. EEG revealed bilateral posterior spikes and spike-waves. Brain MRI showed mild widening of frontal horns and fronto-temporal subarachnoid spaces. Metabolic studies (i.e. urinary organic acids, very long chain fatty acids, chitotriosidase, beta-hexosaminidase A, and beta-galactosidase) were normal. Cerebrospinal fluid neurotransmitter analysis excluded monoamine neurotransmitter disorder. Notably, crying and laughing spells gradually decreased over time, completely disappearing by the age of 9 years. When the patient was 15 years old, she was nonverbal, incontinent, and lacked head and trunk control. She also suffered from chronic constipation. The girl showed dysmorphic features, including long face, thick and medially sparse eyebrows, open bite with widely spaced teeth, large ears with hypoplastic earlobes, bilateral hallux valgus, and long fingers and toes (Fig. 1B). She was microcephalic and presented with diffuse flexion contractures, decreased muscle mass, and severe scoliosis. Neurological examination revealed poor visual fixation and pursuit movements, severe hypotonia, spastic tetraparesis, hand stereotyped movements, and spontaneous clonus of lower extremities. Despite therapy with valproate (30 mg/kg/day) and zonisamide (5 mg/kg/day), recurrent wake and sleep seizures with loss of contact, limb hypertonia, and head rotation persisted. Seizures were usually preceded by a scream and followed by autonomic phenomena (cyanosis, sweating). EEG recording (Fig. 1C) at age 15 years revealed a peculiar bilateral rhythmic spindle activity (13–14 Hz) at eye opening intermixed with interictal spikes and spike-waves prevalent over the vertex and frontocentral areas. Brief seizures featuring limb hypertonia, right head rotation, and impaired contact associated with ictal burst of diffuse high-amplitude sharp waves were also recorded (Fig. 1D). Comparative Genomic Hybridization (CGH)-array, genetic testing for Angelman syndrome, and molecular analysis of *CDKL5*, *MECP2*, *POLG1*, *STXBP1*, *FOXG1*, and *PCDH19* yielded normal results. Family-based whole exome sequencing (WES) identified the *de novo* heterozygous variant c.2443G > T,p.(Gly815Trp) in *GRIN1*.

3. Discussion

GRIN1 is widely expressed throughout the CNS, with 8 different splice variants having distinctive spatiotemporal expression patterns and deeply influencing neuronal function.[1] The structure of Glun1 is complex. This protein consists of a Zinc-binding amino-terminal domain (ATD) responsible for proton inhibition, a ligand-binding domain (LBD) which binds the co-agonists D-serine and Glycine, a transmembrane domain (TMD) forming the ion channel, and an intracellular carboxy-terminal domain (CTD) which interacts with relevant intracellular proteins.[1] As obligatory subunit, GluN1 is required for the proper function of the receptor. NMDAr mediates several essential neurobiological processes, including long-term depression and potentiation. These dynamic synaptic modifications are deeply involved in synaptogenesis, synaptic plasticity, and memory circuits formation. NMDAr signaling further influence neuronal survival. Through the activation of distinct intracellular signaling pathways, the stimulation of synaptic NMDARs exerts neuroprotective effects whereas extrasynaptic receptors mediate cell death promotion.[3]

GRIN1 deficiency is associated with a distinctive and recognizable clinical phenotype.[2] Severe ID/DD with predominant speech involvement are extremely common, whereas epilepsy develops in about two thirds of patients.[4–6]

Seizure types are nonspecific and there is significant heterogeneity in age of onset, EEG patterns, and response to antiepileptic drugs (AEDs) with refractory seizures occurring in about one third of cases. However, some individuals experienced good seizure control with mono- or polytherapy of valproate, vigabatrin, clonazepam, topiramate, levetiracetam, and clobazam.[4] Dystonic eye movements (mostly upward) resembling oculogyric crises and hyperkinetic movements (including chorea, myoclonus, and dyskinesia) are frequently observed. Other common neurologic features are hypotonia, spastic quadriplegia, and cortical visual impairment.[4,5] Patients often exhibit behavioural disturbances such as autistic spectrum disorder (ASD)-like features, bruxism, altered pain perception, inappropriate laughter and crying, hyperventilation, and stereotyped movements with predominant hand involvement (hand-mouthing, hand-washing, and hand-wringing).[2,4] Brain MRI may show minor nonspecific neuroradiologic anomalies and variable cortical atrophy.[4] Interestingly, extensive bilateral polymicrogyria (PMG) resembling tubulinopathy- or GRIN2B-associated dysgyria has been recently described in individuals harbouring *de novo* mutations in critical GluN1 domains.[6]

In line with previous reports, our patients showed a complex electro-clinical phenotype. Patient 1 developed epileptic spasms, dyskinesia, and psychomotor regression at the age of 6 months. Epileptic spasms were effectively controlled with ACTH, but subsequently the child developed cyclic inconsolable crying spells that did not significantly respond to any treatment. Patient 2 suffered instead from refractory epilepsy with variable seizure types, relevant autonomic dysfunction, and a distinctive EEG pattern (Fig. 1C). This girl suddenly developed peculiar and intractable episodes of cyclic laughing, prolonged sleep, and crying.

GRIN1 haploinsufficiency is rare and tolerated in humans, suggesting that the dominant negative effect is the major pathogenic mechanism in *GRIN1* encephalopathy. Most of the patients carry *de novo* heterozygous mutations, clustering within or in close proximity to the highly conserved TMD and leading to ion pore malfunction.[4] Functional consequences of *GRIN1* mutations on the channel properties are more complex than a classical loss of function mechanism.[4,7] Despite the non-negligible phenotypic overlap, some genotype–phenotype correlations are plausible. In particular, milder phenotypes have been associated with pathogenic mutations within the S2 domain of LBD, whereas individuals harbouring homozygous mutations showed suppression-burst EEG, continuous seizure activity, and precocious death.[4] The homozygous *GRIN1* missense mutation c.679G > C (p.Asp227His) has been associated with severe ID and autistic features without epilepsy in two siblings with unaffected heterozygous parents.[7] Furthermore, all the reported patients with extensive bilateral PMG carried gain of function (GOF) variants in the S2 domain and M3 helix of TMD, suggesting that different activation states of NMDAr due to LOF and GOF variants result in distinct clinic-radiological phenotypes.[6] Our patients harboured two distinct pathogenic variants resulting in the replacement of the same amino acid residue, the Glycine 815 in the M4 helix of TDM, and exhibited remarkably overlapping behavioural abnormalities. More specifically, the c.2443G > A, p.(Gly815Arg) variant is described as pathogenic in ClinVar Database and has been previously reported by Lemke et al.,[4] whereas c.2443G > T, p.(Gly815Trp) is a novel variant.

Notably, both our patients showed distinctive circadian rhythm abnormalities characterized by sleep-wake anomalies and cyclic crying or laughing spells. Similar findings have been occasionally reported in other affected individuals,[2] but possible explanations are still lacking. The strict regulation of circadian rhythm is essential for brain homeostasis and peripheral metabolism.[8] In particular, glutamate might represent the main regulator of wakefulness since glutamatergic neurons of the parabrachial-pedunculopontine and supramammillary systems are essential promoters of arousal. Other glutamatergic neurons located in the subcoeruleus and lateral hypothalamic regions are predominantly involved in the generation and promotion of rapid eye movements (REM) sleep, interacting with the parabrachial-pedunculopontine system.[9] Despite sleep-wake neuropharmacology is complex, recent advances suggest that both glutamate and GABA mediate the promotion of arousal and REM sleep, but GABA also promotes non-rapid eye movements (NREM) sleep inhibiting arousal neuronal systems.[9]

This report contributes to expand the phenotypic spectrum of *GRIN1* encephalopathy, suggesting that *GRIN1* should be investigated whether circadian rhythm alterations and cyclic crying or laughing spells are observed in severely intellectually disabled and epileptic children. The evidence that circadian and sleep alterations may act as early pathogenic driver in neurodegenerative disorders[8] further highlights the relevance of these observations and suggest that manipulating glutamate metabolism might represent a valuable therapeutic approach for *GRIN1* encephalopathy in the future.

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Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2019.05.011.

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