

Electro-clinical history of a 5-year-old girl with *GRIN1* gene-related early-onset epileptic encephalopathy: a video-case study

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Abstract

De novo mutations in the GRIN1 gene have been recently reported as the molecular cause of a broad-spectrum early-onset neurological phenotype. Here, we describe a five-year-old girl with an early-onset epileptic encephalopathy associated with an infantile hyperkinetic movement disorder and oculomotor abnormalities. Whole-exome sequencing identified a novel p.Met641Leu de novo variant in the GRIN1 gene as the cause of the phenotype. In silico analysis suggested that the p.Met641Leu variant would alter the gating property of the ion channel, with the involved methionine residue facing towards the ion pore. Long-term systematic video-EEG allowed us to report on the electroclinical history and, specifically, on the semiology of the hyperkinetic movement disorder and oculomotor abnormalities resembling oculogyric crises in our patient. Our findings and a review of the recent literature reinforce the notion of GRIN1-encephalopathy as a recognizable neurological phenotype that should be suspected in early-onset epilepsy associated with hyperkinetic movement disorders.

Key words: GRIN1 gene, epileptic encephalopathy, oculogyric crises, hyperkinetic movements, NMDA receptors

Introduction

Early-onset epileptic encephalopathies (EOEEs) represent a group of severe neurological disorders characterized by refractory epileptiform activity, cognitive regression (or arrest) and a poor prognosis (Sheffer, 2017). NMDA receptors are cationic channels permeable to Na⁺, K⁺, and Ca²⁺ that play a crucial role in excitatory neurotransmission throughout the central nervous system (CNS) (Lemke, 2016). The *GRIN1* gene encodes GluN1 subunit, structurally involved in all NMDA receptors and recently implicated in neurodevelopmental disorders, in association to both *de-novo* and biallelic variants (Lemke, 2016; Rossi, 2017). Although the combination of some clinical features might help in differentiating this condition from other similar epileptic encephalopathies, specific phenotypic featuring of GRIN1-related disorders is still underway. Here, we report on detailed systematic video-EEG and clinical evolution of a patient carrying a *GRIN1 de novo* variant presenting with EOEE and infantile movement disorder.

Case study

The patient was a 5-years-old girl born at 36 weeks of gestation from pregnancy complicated by threatened abortion. At birth, growth parameters were all within normal range. No perinatal pathology was disclosed. At 43 days of life daily seizures appeared characterized by unilateral eyelid and perioral myoclonus, cyanosis affecting the lips, tonic posture of the upper limbs, and clonic jerks of the lower ones, followed by facial rush, eructation and flatulence. These seizures usually appeared after meals. At that time, the neurological examination evidenced poor reactivity to sounds and visual stimuli and axial hypotonia. Auditory and visual evoked potentials were unremarkable. By 12 months of age, the frequency of seizures significantly increased. The girl presented sudden weeping, staring, tonic hyperextension of the upper limbs, mainly when she was falling asleep. Throughout the following months, seizures were daily and prolonged, mainly characterized by eyes deviation and scared gaze, generalized increased tone

and psychomotor agitation. Several antiepileptic drugs were tried such as levetiracetam, valproate, lamotrigine, carbamazepine, barbiturates, hydrocortisone and ACTH, with poor results. At present, the patient presents with several daily oculomotor abnormalities resembling oculogyric crises, most of which are induced by visual fixation (see video sequence).

Global psychomotor delay was observed early in infancy. The patient never achieved head control or the ability to sit or stand and did not gain any language skills. Severe axial hypotonia and distal dystonic posturing (mainly affecting the fists and ankles) were observed since the first months of life. She presented with choreoathetoid and stereotyped movements (see video sequence).

At the last examination, at five years of age, she still presented with poor reactivity to sounds and visual stimuli. The patient showed severe axial hypotonia with absent head and trunk control, and feeding difficulties. Dystonic posturing of the hands (claw hands), equinovarus deformity and brisk osteotendinous reflexes were evident at the neurological examination. Brain MRI scan, performed at 2 months and 3 years of age, revealed non-progressive paratrigonal white matter hyperintensity with partial involvement of tapetum and left hippocampal atrophy (Figure 1 A-D). Extensive diagnostic and metabolic work-up was unrevealing, and the patient also underwent molecular investigation including array comparative genome hybridization (array-CGH) and panel sequencing for 96 EOEE-causing genes that were both reported as normal.

Neurophysiological investigations

Normal EEG background activity was recorded at the onset of seizures with isolated and sporadic unusual spiky theta waves, mainly during the quiet sleep (see video sequence). A neonatal electrical pattern persisted during sleep up to 2 months of corrected age (Figure 2A). The onset of deterioration of EEG activity occurred at the age of 2 years with appearance of unusual slow, low-voltage activities with superimposed high-voltage spikes on bilateral central

regions, with no evidence of spindles during stage 2 of sleep (Figure 2, B-D). At 4 years of age, the EEG during sleep showed recurrent, interictal, high-voltage, bilateral, spike-wave discharges, at 1-1,5 Hz, on bilateral centro-temporal regions followed by brief sequences of slow delta activities, interspersed with brief tracts of diffuse background slowing, with a multifocal 'quasi-periodic' pattern (Figure 2, E).

Whole exome sequencing

Clinical whole-exome sequencing (WES) was performed for the proband and her unaffected parents (Figure 3). The Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer instructions. Libraries were sequenced in an Illumina HiSeq3000 using a 100-bp paired-end reads protocol. Sequence alignment to the human reference genome (UCSC hg19), and variants call and annotation were performed using an in-house pipeline as described elsewhere (Mencacci, 2016). The raw list of single nucleotide variants (SNVs) and indels was then filtered. Only exonic and donor/acceptor splicing variants were considered. In accordance with the pedigree and phenotype, priority was given to rare variants [$<1\%$ in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that fit a recessive or a *de-novo* model and are located within genes previously associated with EOEE.

The *de novo* *GRIN1* variant identified by WES in the proband (c.1921A>T; p.Met641Leu) was confirmed by traditional Sanger sequencing. The detailed conditions for sequencing analysis are available upon request.

Discussion

We report the clinical and video-EEG history of a patient presenting with EOEE, severe psychomotor delay, and a complex hyperkinetic movement disorder with stereotypies, carrying a *de-novo* (novel) p.Met641Leu heterozygous variant in the *GRIN1* gene, as detected by WES trio analysis. In contrast to the majority of the reported cases, although our patient presented with neonatal-onset seizures with an early immature and atypical EEG pattern, onset of

multifocal “quasi-periodic” discharges was not evidenced before the age of five years. Choreo-athetoid movements and motor stereotypies were mostly evident within infancy, but became less evident at the last visit at five years, when oculogyric crises were predominant. Global EEG background activity later became disorganized with loss of physiological sleep architecture at the age of five years. However, the onset of a multifocal “quasi-periodic” pattern and seizure worsening did not induce any neurodevelopmental and/or epileptic course modifications. According to these findings and in the light of the newly updated International League Against Epilepsy (ILAE) Classification, GRIN-1 encephalopathy might be defined as “developmental and epileptic encephalopathy” (Scheffer et al., 2017). The role of NMDAR is increasingly emphasized in neurodevelopment (Lemke, 2016; Chen, 2017; Zehavi, 2017). Moreover, *GRIN1* variants are likely to affect function of both NMDA and dopamine D1 receptors, thus likely explaining the associated extrapyramidal symptoms (Lee, 2002). To date, more than 30 patients with *GRIN1* mutations and epilepsy have been reported; the majority with *de novo* heterozygous and 3 unrelated families with inherited homozygous variants (Lemke, 2016; Rossi, 2017; Ohba 2015; Zehavi, 2017). Despite the core clinical phenotype includes developmental delay, muscular hypotonia, hyperkinetic movements (including chorea and dyskinesia) and oculomotor abnormalities (Ohba 2015; Lemke, 2016; Zehavi, 2017), some features such as epilepsy and degree of cognitive and behavioural dysfunction, appear to be relatively heterogeneous. Although 19 previous patients presented with epilepsy with a different age at onset and severity, only four patients were reported to present with unclassified EOEE according to Ohba et al. (2015) and three presented with infantile spasms according to Lemke et al. (2016).

Non-specific neuroradiological findings in previous patients, as in ours, did not seem to influence clinical severity.

As in our case, *de-novo* pathogenic variants mostly cluster in the transmembrane domains of the gene, however, no clear-cut genotype-phenotype correlation has so far ascertained. A variant involving the same Met641 residue (p.Met641Ile) has been previously reported in a 14 year-old-

male showing a different phenotype from those observed in our case. Breath-holding attacks were the main seizures type. Abnormal eye movements, tonic posture of unilateral limbs, severe intellectual disability and hyperreflexia were further phenotypic features. Focal epileptiform discharges were evident on the EEG from the onset of the seizures, at the age of 3 months (Lemke, 2016). The overlapping phenotypes of several EOEEs may complicate the possibility of their systematic categorization. Detailed reports of electroclinical features and evolution, as well as neurodevelopmental outcomes are mandatory to define novel syndromic entities and may further support clinical work- up, parents counseling and treatment options.

Disclosures

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

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Figures

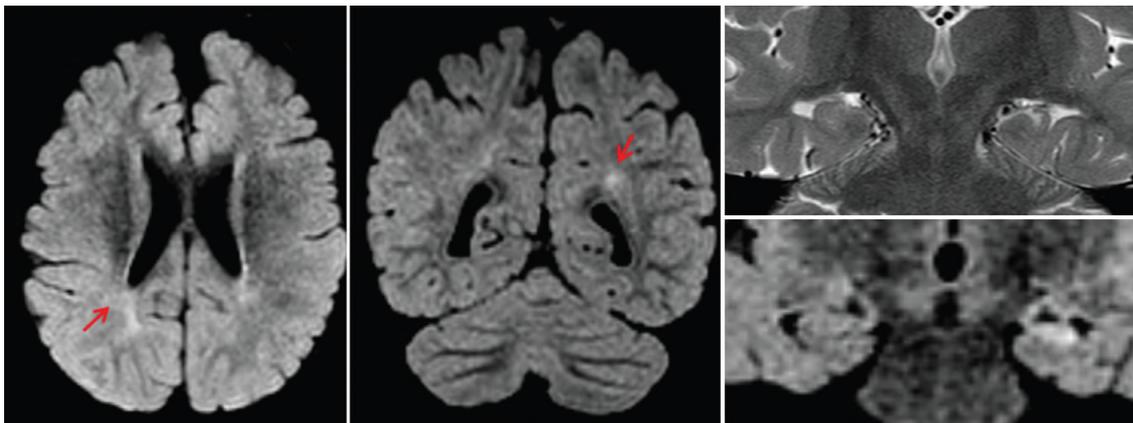


Figure 1:

Axial (a) and Coronal (b) Fluid-Attenuated Inversion Recovery (FLAIR) MRI images. Paratrigonal white matter hyperintensity with partial involvement of tapetum (red arrows). Coronal T2 (c) and FLAIR (d) MRI images. Left hippocampal atrophy with increased T2 signal.

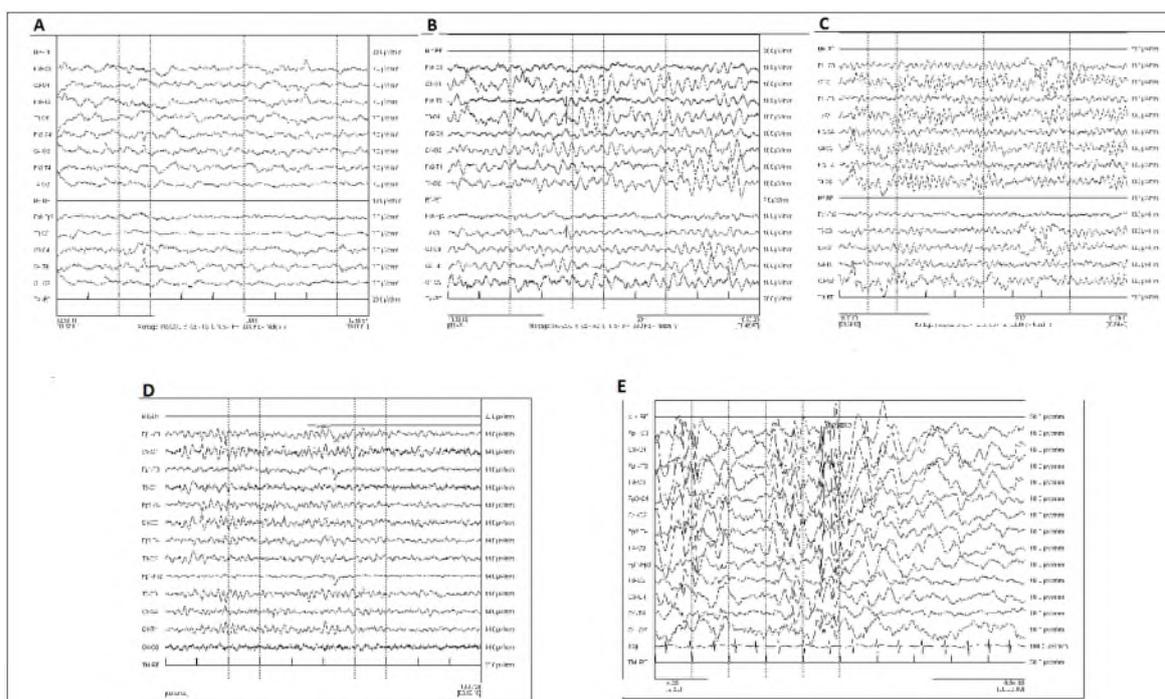


Figure 2:

A-E. Evolution of EEG in the patient: (A) quiet sleep state at 46 days of life, with diffuse and continuous slow, low-medium voltage activity with recurrent superimposed theta sharp waves and spindle-like sequences on right fronto-central regions; (B) awake/drowsy state at three months of age, with recurrent, high-voltage deltawaves on bilateral occipital regions, mostly asynchronous; (C) awake/drowsy state at 12 months of age, with recurrent, synchronous, high-voltage delta waves on bilateral posterior regions; (D) during sleep at two years of age, showing diffuse slow, low-voltage activity with isolated high-voltage spikes on bilateral central regions, with no evidence of spindles; and (E) during sleep at four years of age, showing recurrent interictal high-voltage, bilateral spike-wave discharges at 1-1.5 Hz on bilateral centro-temporal regions, followed by brief sequences of slow delta activity, interspersed with brief tracts of diffuse background slowing with a “quasi-periodic” pattern. The physiological figures of Stage 2 sleep (spindles and vertex spikes) are poorly organised.

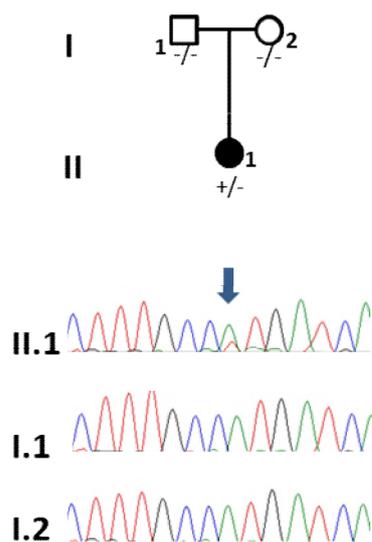


Figure 3:

Clinical whole-exome sequencing (WES) for the proband (II-1) and her unaffected parents (I-1, I-2).

Video Legend

Video 1: A, EEG recorded at the age of 1 month and 22 days during sleep shows diffuse and continuous slow, low-medium-voltage activity with recurrent superimposed theta sharp waves and spindle-like sequences on R, fronto-central regions; **B**: oculogyric movements, sucking automatisms, choreoathetoid and stereotyped movements at the four limbs during awake concomitant with posterior medium-voltage, 4-5 Hz activity with no epileptiform discharges (2 years and 5 months); **C**: persistency of oculogyric, choreoathetoid and stereotyped movements associated to diffuse slowing of the background activity and artifacts, no clear epileptiform discharges **D**: oculogyric crises (R upward eye deviation, converging movements, blinking with pupil dilation and some opsoclonic jerks), mostly exerted by visual fixation, are associated to high-voltage, bi-triphasic, spikes and polyspikes on R centro-temporal-occipital regions (5 years and 4 months).

Short questions:

1. May GRIN-1 be related to early-onset epileptic encephalopathy?

GRIN1 could be taken into account in a diagnostic work-up of early-onset epileptic encephalopathies.

2. What type of seizures could be seen in GRIN1-related epileptic encephalopathy?

The most common GRIN-1 –related seizures are focal ones and involve ocular and facial districts.

3. Can GRIN1 gene be related to infantile movement disorders?

Yes, GRIN1 gene can be related to movement disorders in patients with severe infantile encephalopathies.