Short communication

**GRIA3 missense mutation is cause of an x-linked developmental and epileptic encephalopathy**

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**ABSTRACT**

Purpose: GRIA3, encoding subunit 3 of glutamate ionotropic AMPA receptor, is associated with X-linked intellectual disability (ID), dysmorphic features, and non-syndromic epilepsy. We aimed to characterize electro-clinical features of patients with GRIA3 variants.

Methods: We report a patient carrying a hemizygous missense variant c.2359 G > A (p.Glu787Lys) in GRIA3 gene. Following a literature search, we also reviewed clinical, electrophysiological, radiological, and genetic features of 19 patients with GRIA3 mutations.

Results: This 26-month-old boy had developmental delay, early onset refractory myoclonic epilepsy, and non-convulsive refractory status epilepticus. In published reports, epilepsy was in 6 of 19 patients carrying different genotypes, though epilepsy and electroencephalogram features were not completely defined. Out of the 6 patients, one presented with generalized tonic-clonic seizures, two with myoclonic and clonic events (one also presented with epileptic spasms), and one with atypical absences and myoclonic jerks. Information on type of epilepsy was unavailable for 3 cases. Epilepsy onset was early in life and there was potential tendency for myoclonic/clonic events. The epilepsy was difficult to treat and prognosis is poor. Severity of ID ranged from mild to severe and was variably associated with bipolar affective disorder and autistic spectrum disorders. Other neurological features included hypotonia, asthenic body habitus with poor muscle bulk, and hyporeflexia.

Conclusion: Our report expands knowledge on the electro-clinical and molecular spectrum of GRIA3 variants. Larger investigations will better define the prevalence of epilepsy, the epileptic phenotype, and syndromic features underlying GRIA3 variants.

1. Introduction

Ionotopic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors mediate fast excitatory synaptic transmission in the central nervous system (CNS). AMPA receptors are crucial for brain development and modulate activity-dependent synaptic plasticity [1].

GRIA3, encoding subunit 3 of the glutamate ionotropic AMPA receptor (GluR3), has been identified as a candidate gene for X-linked intellectual disability (ID) in males [2]. To date, 19 cases have been reported (Table 1). GRIA3 mutations are hypothesized to cause a loss in ionotopic function of GluR3 and loss of channel current [3]. GRIA3 genetic variants have been associated with a clinical phenotype characterized by moderate ID, dysmorphic features, and epilepsy in a third of cases [2–11].

We report the clinical characteristics of a case with developmental and epileptic encephalopathy (DEE) due to a pathogenic missense mutation. We also reviewed the clinical features of all previously reported cases with GRIA3 genetic variants to determine the clinical phenotype that might aid identification of future mutated patients. We focused on epilepsy phenotype because this was poorly reported in previous cases and well-documented in our case.

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Table 1
Demography and clinical findings.

<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Sex</th>
<th>Inheritance</th>
<th>Age at last F-U</th>
<th>Development</th>
<th>Dysmorphic features</th>
<th>Additional clinical features</th>
<th>Epilepsy</th>
<th>Electrophysiological tests</th>
<th>MRI findings</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geez et al. 1999</td>
<td>F</td>
<td>De novo</td>
<td>20y</td>
<td>ID, bipolar affective disorder</td>
<td>n.a.</td>
<td>n.a.</td>
<td>2 GTCS (18 m)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>t(X;12)(q24; q15)</td>
</tr>
<tr>
<td>Wu et al. 2007</td>
<td>M</td>
<td>Mother</td>
<td>n.a.</td>
<td>Moderate ID</td>
<td>n.a.</td>
<td>Poor muscle bulk, hyporeflexia</td>
<td>no</td>
<td>n.a.</td>
<td>n.a.</td>
<td>del 0.4 Mb</td>
</tr>
<tr>
<td>Wu et al. 2007</td>
<td>M</td>
<td>Mother</td>
<td>n.a.</td>
<td>Moderate ID, ASD</td>
<td>n.a.</td>
<td>Macrocephaly</td>
<td>Seizures with myoclonic jerks</td>
<td>n.a.</td>
<td>n.a.</td>
<td>c.2497 G &gt; C (p.Gly833Arg)</td>
</tr>
<tr>
<td>Wu et al. 2007</td>
<td>M</td>
<td>Mother</td>
<td>n.a.</td>
<td>Moderate ID</td>
<td>n.a.</td>
<td>Asthenic body habitus, poor muscle bulk, distal muscle weakness, hyporeflexia</td>
<td>No</td>
<td>n.a.</td>
<td>n.a.</td>
<td>c.2117 T &gt; C (p.Met706Thr)</td>
</tr>
<tr>
<td>Wu et al. 2007</td>
<td>M</td>
<td>Mother</td>
<td>n.a.</td>
<td>Moderate ID</td>
<td>n.a.</td>
<td>Asthenic body habitus</td>
<td>No</td>
<td>n.a.</td>
<td>n.a.</td>
<td>c.1891C &gt; A (p.Arg631Ser)</td>
</tr>
<tr>
<td>Wu et al. 2007</td>
<td>M</td>
<td>Mother</td>
<td>n.a.</td>
<td>ID</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>c.1394 G &gt; A (p.Arg450Gln)</td>
<td></td>
</tr>
<tr>
<td>Chiyonobu et al. 2007</td>
<td>M</td>
<td>Mother</td>
<td>4y7m</td>
<td>Severe DD (DQ = 25)</td>
<td>Short stature, hypertelorism, epicanthal fold, short neck</td>
<td>Right inguinal hernia</td>
<td>No</td>
<td>n.a.</td>
<td>n.a.</td>
<td>dup 0.498-kb</td>
</tr>
<tr>
<td>Bonnet et al. 2009</td>
<td>M</td>
<td>Mother</td>
<td>20y*</td>
<td>Mild ID (IQ = 59)</td>
<td>n.a.</td>
<td>Inguinal hernia</td>
<td>No</td>
<td>n.a.</td>
<td>Normal</td>
<td>dup 0.275-kb</td>
</tr>
<tr>
<td>Bonnet et al. 2009</td>
<td>M</td>
<td>Mother</td>
<td>20y*</td>
<td>Moderate ID</td>
<td>Narrow palate, prominent incisors, short philtrum, uplift of ear lobules</td>
<td>n.a.</td>
<td>No</td>
<td>n.a.</td>
<td>Retrocerebellar cyst</td>
<td>dup 0.275-kb</td>
</tr>
<tr>
<td>Philippe et al. 2013</td>
<td>M</td>
<td>Mother</td>
<td>24y</td>
<td>Moderate ID (IQ = 50)</td>
<td>Malar flatness, thick vermilion, lower palpebral eversion, prominent supraorbital ridges</td>
<td>Hypotonia, pain insensibility, scoliosis, testicular ectopia</td>
<td>No</td>
<td>n.a.</td>
<td>Thin CC, Atrophy of the last 2 lobules of the cerebellar vermis</td>
<td>dup 1.2-Mb</td>
</tr>
<tr>
<td>Philippe et al. 2013</td>
<td>M</td>
<td>Mother</td>
<td>4y6m</td>
<td>Mild ID</td>
<td>Epicantic folds, malar flatness, thick vermilion, hypotonic facies</td>
<td>Hypotonia, joint laxity, valgus pes planus, genu recurvatum, micropenis</td>
<td>No</td>
<td>n.a.</td>
<td>Thin CC (posterior part)</td>
<td>dup 5.02-Mb</td>
</tr>
<tr>
<td>Philips et al. 2014</td>
<td>M</td>
<td>n.a.</td>
<td>57y</td>
<td>Severe ID, ASD</td>
<td>Short stature, brachycephaly, deep set eyes, prominent supraorbital ridges</td>
<td>n.a.</td>
<td>Yes</td>
<td>n.a.</td>
<td>c.1888 G &gt; C (p.Gly630Arg)</td>
<td></td>
</tr>
<tr>
<td>Philips et al. 2014</td>
<td>M</td>
<td>Mother</td>
<td>36y*</td>
<td>Severe ID</td>
<td>Short stature, brachycephaly, deep set eyes, prominent supraorbital ridges</td>
<td>Hydronephrosis, renal arcuatus, malposition of feet</td>
<td>Yes</td>
<td>n.a.</td>
<td>c.1888 G &gt; C (p.Gly630Arg)</td>
<td></td>
</tr>
<tr>
<td>Philips et al. 2014</td>
<td>M</td>
<td>Mother</td>
<td>36y*</td>
<td>Severe ID, ASD</td>
<td>Short stature, brachycephaly, deep set eyes, prominent supraorbital ridges</td>
<td>n.a.</td>
<td>Yes</td>
<td>n.a.</td>
<td>c.1888 G &gt; C (p.Gly630Arg)</td>
<td></td>
</tr>
<tr>
<td>Allen et al. 2016</td>
<td>F</td>
<td>De novo</td>
<td>7y</td>
<td>Severe ID</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Clonic ad tonic seizures (6 w); infantile spasms (4 m), focal seizures</td>
<td>Normal</td>
</tr>
<tr>
<td>Cherot et al. 2017</td>
<td>M</td>
<td>Mother</td>
<td>n.a.</td>
<td>ID</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>(continued on next page)</td>
</tr>
</tbody>
</table>
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2. Case report

The patient presented as a 26-month-old boy – first child of non-consanguineous parents without relevant personal or familiar medical antecedents. He was born at 38 weeks of gestational age by uncomplicated delivery. Developmental delay was evident from the first months of life, mainly characterized by axial hypotonia: he had poor eye contact, did gain head control at the age of 6 months, but never acquired other motor skills.

All early investigations were normal: metabolic screening of blood, urine, and cerebrospinal fluid (CSF); visual and brainstem-evoked potential; audiometry; electroretinography; electro-neurography; and electromyography. Abdominal ultrasound was normal.

Hypothyroidism was diagnosed at the age of 12 months and substitution treatment was started. Brain MRI also performed at the age of 12 months was normal. Electroencephalogram (EEG) showed the presence of severe disruption of background activity and multifocal epileptiform abnormalities intermingled with high-voltage slow waves. Therefore, even though clinical seizures were absent, levetiracetam was started. After 2 months, no change in EEG was seen, so levetiracetam was replaced with clobazam and valproic acid.

Aged 16 months, he presented with a global deterioration: worsening of vigilance, feeding difficulties, and asthenic body habitus with poor muscle bulk. EEG revealed a pattern characterized by subcontinuous diffuse epileptiform abnormalities with a higher amplitude over the bilateral frontal regions (Fig. 1A-B). No obvious clinical events were recorded. Adrenocorticotropic hormone (ACTH) 0.1 mg/day was administered (limited to 10 days because of sepsis), but without effect. After ten days, clinical status was characterized by unresponsiveness with fluctuating eyelid myoclonia and jerks in the distal segments of limbs. EEG showed a non-convulsive status epilepticus (NCSE) with continuous diffuse epileptiform abnormalities (Fig. 1D). The patient was treated in our intensive care unit (ICU) with midazolam, ketamine, sodium thiopental, and propofol. NCSE lasted 20 days, after which clinical conditions improved.

At the last follow-up (aged 26 months), cognitive assessment showed a severe developmental delay with severe axial hypotonia – sitting position and walk had not been acquired – and hyporeflexia. Obtaining visual attention was persistently difficult language was limited to non-verbal sounds, and mild dysmorphic features were identified (Table 1). Gastrostomy had been performed at the age of 2 years. Video-EEG recording showed a slow background activity with recurrent high-voltage slow waves intermingled with epileptiform abnormalities, predominantly over the frontal regions. Daily multiple atypical absences associated with eyelid myoclonia were disclosed and he was treated with topiramate. He never experienced major motor seizures.

Whole exome sequencing revealed the hemizygous missense variant c.2359 G > A (p.Glu787Lys) in the GRIA3 gene (NM_000828) (Fig. 1E, G). Inherited from his healthy mother, this variant was not present in a public database (gnomAD v2.1.1) but was predicted as pathogenic by bioinformatic tools (SIFT: deleterious; PolyPhen: probably damaging; Condel: deleterious; CADD:32) and affected an amino acid that is highly conserved among eukaryotes (Fig. 1F,H).

Based on this genetic finding, we trialed adjunctive administration of a selective non-competitive antagonist of AMPA receptors (perampanel 4 mg/day) for one month, but no change in EEG or clinical condition was observed.

3. Discussion

Since its first description, 19 cases of GRIA3-associated, X-linked ID with dysmorphic features and non-syndromic epilepsy have been reported previously to our case. The genotype was characterized by different chromosomal abnormalities: balanced translocation (n = 1), deletion (n = 1), duplications (n = 5), and missense GRIA3 genetic variants (n = 8) [2-11].
We have described the electro-clinical phenotype of a patient with a severe DEE due to a missense \textit{GRIA3} genetic variant. Clinical distinction between worsening of his epileptic encephalopathy and the NCSE was difficult to discern. Nevertheless, our patient had worsened vigilance and the appearance of eyelid myoclonia, which was diagnosed as NCSE from the EEG characteristics \cite{12} and treated with anesthetic drugs. Clinical findings were similar to what has been reported as \textquotedblleft myoclonic status in non-progressive encephalopathies\textquotedblright{} \cite{14}.

Epilepsy was reported in six out of 19 previously reported patients, representing three different missense variants \cite{3,8,9} and one balanced...
translocation [2]. Epilepsy in these reports was mentioned only briefly and with few details. Concordant with our patient, age at onset of epilepsy was early in life in one previously reported case [9]. Seizure semiology is difficult to describe within cases; however, potential tendency for myoclonic/clonic events (2 out of 6 patients) [3,9] is additionally supported by our patient.

SE was not reported in previous cases. Even after resolution of SE, our patient continued to present with subtle jerks of limbs associated with diffuse spikes and slow waves. Myoclonus occurs in other syndromic conditions; myoclonus is a significant finding of Angelman Syndrome, usually with later onset and appears to increase with age. Some clinical and EEG features we have observed – such as duration (lasting from several seconds to several minutes, most often with near-constant myoclonus), resistance to anti-seizure medications, occurrence of diffuse spikes and slow waves – are features seemingly in common with Angelman Syndrome [10]. The one patient previously reported also had epileptic spasms [9], but no EEG was documented. Generally, differentiating between subtle epileptic spasms and erratic myoclonus can be challenging. In this regard, analysis of the paroxysmal event with the support of polygraphic recordings is suggested for patients with Angelman Syndrome and is possibly equally applicable for this phenotype.

Few data are available on EEG or prognosis. EEG in our case was severely abnormal, with absent physiologic background activity. Abnormal EEG persisted during the follow-up, with bi-frontal epileptoform abnormalities and multifocal slow waves, which continued irrespective of antiseizure medications (ASMs). Steroid treatment (ACTH) was without observable efficacy. Ketogenic diet has not yet been tried and reported, though it might have been a good therapeutic option in this electro-clinical context.

A possible role of GRIA3 in epileptogenesis might be based on the involvement of AMPA receptors in glutamatergic transmission and fast excitatory synaptic transmission in the cerebral cortex [1,3,15]. Trial of perampanel (the only available drug active on AMPA receptor) failed in our patient. We did not perform functional studies regarding our patient’s variant; hence, we do not know if the mutation could cause a gain or loss of function, or if perampanel would have been expected to restore the function of the receptor. Electro-clinical features associated with GRIA3 variants are still poorly reported in literature. The electro-clinical phenotype characterized by diffuse slow waves and multifocal abnormalities associated with myoclonic jerks could involve AMPA receptors, but this requires verification in larger series.

Mild to severe ID was consistently reported in all cases, including our patient; however, the severity of ID has widely varied and seems to be independent from genotype. ID has been variably associated with bipo lar affective disorder [2] and autistic spectrum disorders [3,5,8]. Other neurological features (with or without epilepsy) were also reported previously, such as marked hypotonia, asthenic body habitus with poor muscle bulk, hyporeflexia, and sleep disturbances [3,8,10]. We performed a comprehensive electrophysiological study, including electromyography and electromyography, which yielded nothing remarkable despite the presence of hyporeflexia.

To conclude, we report the electro-clinical phenotype of a patient with a DEE due to a novel missense mutation of GRIA3 inherited from his healthy mother. This widens the phenotype of this rare neurologic condition. The epileptic phenotype of this and the few other reported cases is characterized mainly by brief myoclonic and clonic movements and atypical absence seizures without prominent motor manifestations. Such seizure semiology can be misdiagnosed as the abnormal movements usually seen in severely disabled children; hence, we hypothesize that epilepsy will be underdiagnosed in GRIA3 patients unless video-polygraphic recordings are systematically performed. Additionally, this gene could be included in existing NGS panels for patients with epilepsy with suspected genetic etiology. Further investigations of a larger number of patients is still required to better define the prevalence of epilepsy, the main features epileptic phenotype, and syndromic features underlying GRIA3 genetic variants.

4. Disclosure

The study was conducted according to the local ethics committee. Written consent was obtained from patients’ parents/guardians.

None of the authors has any conflict of interest to disclose. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.seizure.2020.08.032.

References


