Article type: Case report

Title: Paroxysmal limb dystonias associated with *GABBR2* pathogenic variant: a case-based literature review

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Abstract: (214/250 words max)

Background. *De novo* mutations in the *GABBR2* gene have recently been reported to be associated with a form of early-infantile epileptic encephalopathy (EIEE59; OMIM# 617904), as well as a Rett syndrome-like (RTT) disorder defined as a neurodevelopmental disorder with poor language and loss of hand skills (NDPLHS; OMIM# 617903).

Methods. We describe a new pediatric case of *de novo GABBR2* mutation manifesting as RTT, epilepsy, generalized hypotonia and paroxysmal limb dystonia.

Results. A 11-year-old girl, born to non-consanguinous parents after an uneventful pregnancy, had developmental delay and generalized hypotonia. At age 3.5 months she presented with infantile spasms with an electroencephalographic pattern of hypsarrhythmia. After treatment with clonazepam and prednisolone, she was seizure-free with a slow background electrical activity. Magnetic resonance imaging was normal. Paroxysmal dystonic posturing of the extremities, especially the upper limbs, have been observed since the age of 3 years. Motor stereotypes, non-epileptic episodes of hyperventilation and breath-holding were also reported. The girl suffered from feeding difficulties that necessitated the gastrostomy insertion at the age of 8. Whole exome sequencing revealed a *de novo* pathogenic variant, previously reported in the ClinVar database (ID 488080) (NM_005458:c.G2077T:p.G693W).

Conclusion Paroxysmal limb dystonias, especially in the context of neurodevelopmental disorder featuring epilepsy, generalized hypotonia and RTT-like features should lead to the suspect of *GABBR2* mutations.

Keywords: GABBR2; dystonia; WES; early infantile epileptic encephalopathy; Rett syndrome (3 to 9 max)

Introduction:

GABAB receptor (GABABr) is an inhibitory metabotropic receptor belonging to the superfamily of G-protein coupled receptors [1]. GABABr is assembled by the heterodimeric interaction of the intracellular C-terminal tails of the two subunits, encoded by *GABBR1* and *GABBR2* [1]. *GABBR2* encodes a protein of 941 amino acids, arranged into 7 transmembrane (TM) domain proteins and it shares approximately one-third of the aminoacid sequence with GABBR1 [2]. *De novo* mutations in the *GABBR2* gene have recently been described as causing a form of early-infantile epileptic encephalopathy (EIEE59; OMIM# 617904), as well as a Rett syndrome (RTT) -like disorder defined as a neurodevelopmental disorder with poor language and loss of hand skills (NDPLHS; OMIM# 617903). It has been proposed that the phenotypic features may depend on the site of the genetic mutation, in particular the TM domain involved [3,4]. In addition to intellectual disability (ID), epilepsy and autism spectrum disorder (ASD), hypotonia and hyporeflexia have also been described in association with *GABBR2* mutations. The proposed disease mechanism is under-activation at the neuromuscular junction (NMJ) and/or in spinal motor control centers [3]. Interestingly, GABABr is the molecular target of baclofen, which act as an agonist of the receptor and it is widely used in neurology practice as a muscle relaxant and antispastic drug [5]. Of note, individual cases carrying *GABBR2* variants associated with dystonic posturing have been reported before [4,6]; although, the underlying genotype-phenotype correlations for this rare genetic condition remain limited. We report a new case of a young girl with a heterozygous *GABBR2* mutation, manifesting as RTT, epilepsy, generalized hypotonia and paroxysmal limb dystonias.

Methods

We describe a girl with broad neurodevelopmental impairment and paroxysmal dystonic features, in which whole-exome sequencing (WES) identified a *de novo GABBR2* mutation as the likely genetic cause of her disease. We performed a literature review of patients carrying *GABBR2* variants associated with dystonic features and compared the clinical features among these individuals.

Results

Patient description

This 11-year-old Maltese girl presented at 3.5 months with infantile spasms (IS). She was born at term after an uneventful pregnancy and required nasogastric tube feeding for slow feeding in the first 3 days of life. At 3 months, clinical examination showed truncal and limb hypotonia, head lag, poor visual following, intermittent nystagmus and a convergent squint. Facies appeared normal while growth parameter plotted on the 50th percentile (P50) with an occipital-frontal head circumference

(OFC) of 42 cm (P50). At this stage, she also had signs of early aspiration with a lower respiratory tract infection. Electroencephalography (EEG) confirmed hypsarrhythmia which responded to prednisolone and clonazepam and by 2 weeks EEG had improved apart from slow background activity. Magnetic resonance imaging (MRI) / magnetic resonance spectroscopy (MRS) brain scans were unremarkable. At follow-up, she was seizure-free by 2 years 5 months but continued to show signs of a profound global developmental delay with generalised hypotonia in the setting of brisk reflexes. Head circumference and weight continued to track along the P50 centile. By 3 years of age, abnormal involuntary limb movements developed characterized by paroxysmal dystonic posturing of the arms more than the legs and other hand stereotypic movements. Over the years, this was associated with progressive loss of the voluntary use of her hands. By 7 years of age, she developed distressing episodes of hyperventilation and breath-holding followed by behavioural arrest and head drops. Repeated ictal EEGs excluded epileptiform activity and an atypical presentation of Rett syndrome was suspected. Feeding difficulties became more evident at this stage characterized by food refusal and early morning retching. A percutaneous endoscopic gastrostomy was inserted at 8 years of age and gastroscopy revealed eosinophilic esophagitis. Family history was unremarkable; her parents are non-consanguineous and her older sibling are healthy. She has been investigated and array-CGH, metabolic screen including plasma/ urine amino acids, urine organic acids, acylcarnitine, lactate, white blood cell enzymes, creatine, MRS and neurotransmitter studies, muscle biopsy, electromyography and nerve conductions studies were normal. Whole exome sequencing was performed in the proband and her parents revealed a *de novo* variant previously reported in the ClinVar database (ID 488080) (NM_005458:c.G2077T:p.G693W).

Discussion

Genetic developmental disorders with or without regression include a variety of monogenic conditions, including RTT and RTT-like disorders, with expanding clinical differential diagnosis, genetic heterogeneity, and associated disease mechanisms [7–9].

GABBR2 mutations were first detected in 2014 in two individuals affected with neurodevelopmental impairment (with RTT-like features) or DEE who underwent WES as part of their diagnostic workup [10]. Subsequently, *de novo* mutations in *GABBR2* have been identified in cohorts of individuals affected with ID [11,12], ASD [13,14] and (refractory) epilepsy [15,16]. These findings were followed by several reports of individuals affected with *GABBR2* variants. To the best of our knowledge, 17 *GABBR2* heterozygous variants have been reported so far in the literature (Table 1), including a recurrent missense variant (c.1699G>A; p.A567T) which affects a highly conserved residue within the TM3 domain [4,6,11–13]. ID is a constant feature of *GABBR2* mutation carriers,

and it is often observed in the context of RTT and ASD presentations. MRI and EEG are usually unremarkable in these patients or display unspecific features [4]. Hypotonia and ataxia can be part of the neurological phenotype of the disease in some cases. Dystonic attacks may involve either the lower or the upper limbs [6] and, rarely, are associated with abnormal ocular movements [4].

Epilepsy is more peculiar to those individuals carrying variants within the TM6 domain (p.S695I, p.I705N, p.G693W). However, TM6 variants associated with RTT-like features in the absence of clinically evident epilepsy have also been described in some cases [17]. Seizures are often polymorphic in these patients and can include IS (and hypsarrhythmia at EEG), as well as focal and generalized seizures. In our case, the EEG pattern improved after using a combined clonazepam and steroid (prednisolone) therapy. Interestingly, Chin et al. showed EEG normalization after vigabatrin treatment, a drug that is known to increase the bioavailability of GABA as a neurotransmitter [18].

The first experimental studies investigating *GABBR2* mutations were carried out by Yoo et al. [4], initially using *in vitro* and *in vivo* approaches. *In vitro* studies suggested disease mechanisms in *GABBR2*-related RTT- vs. DEE- phenotypes may arise by the effect of distinct mutations within the TM3 and TM6 domains, respectively. These mutations may exert specific effects on the residual receptor activity, resulting in peculiar clinical phenotypes.

The p.A567T variant (involving TM3 and classically associated with RTT-like phenotypes) showed an agonist-induced reduction in the receptor activity of approximately 30% when compared to the wild-type receptor. This reduction resulted in a further increase in the DEE-related mutant receptors p.S695I and p.I705N (variants located in the TM6 domain). These findings were also confirmed in animals model (*i.e.*, tadpoles) featuring seizure-like behaviour and swimming ability impaired as the result of RTT- and DEE-related *GABBR2* mutations. Interestingly, the addition of baclofen was able to partially reverse these impairments *in vivo*. The authors concluded that mutations in TM6 (DEE-related are associated with more severe phenotypes due to structural instability, whereas mutations in TM3 might be more likely to be involved in activation pathways. Vuillaume et al. have reported a novel mutation, A707T, located in TM6 but associated with RTT phenotype [19]. In their functional study, using an *in vitro* model, they demonstrated increased basal activity of the mutated receptor in p.A707T and the three variants previously investigated by Yoo et al. and therefore suggested a novel underlying pathogenetic mechanism, due to constitutive activity of the mutated receptor.

To date, no functional studies have been carried out to explain the possible pathogenesis of dystonic posturing. Recently, Sun et al. have found a *GABBR2* variant associated with cervical dystonia using next-generation sequencing [20]. The authors conclude that future studies will be needed to confirm this finding and the disease association of the *GABBR2* gene with (paroxysmal) dystonic posturing.

Conclusions

We report the first individual carrying a *GABBR2* pathogenic variant associated with generalised hypotonia with paroxysmal limb dystonic episodes as well as RTT-like and infantile-onset DEE phenotypes. We did not perform functional studies to characterize cellular and sub-cellular effects of this specific variant. Importantly, the *GABBR2* gene is ubiquitously expressed in the central nervous system [2]. As suggested by Yoo et al. in their reply to Vuillaume's letter, the pathogenetic mechanisms may be multiple and functionally involve other genes even through neuronal circuits [19]. Future studies are encouraged to investigate the functional aspects of the mutated *GABBR2* receptor subunit and the possible role of baclofen and other direct and indirect GABA-agonists. The prevalence of variants in *GABBR2* within clinical cohorts (or genomic datasets) of individuals with molecularly undiagnosed (paroxysmal) dystonic posturing (especially on the background of neurodevelopmental impairment and/or epilepsy) should also be explored.

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Conflict of interest
The authors declare that they have no conflict of interest.

Data statement
The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

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Figure legend

Fig.1. Iconography, protein localisation and family tree of our patient with pathogenic variant in *GABBR2*.

A) Patient's appearance at the age of 2 (left panel), 4 (middle panel), and 7 (right panel) years. Note the dystonic posturing and the convergent strabismus.

B) GABBR2 protein structure and localisation of the known variant from the literature associated with similar phenotypes.

C) Pedigree of the family.

Table 1. Previously published cases of *GABBR2* and genotype-phenotype correlation.

Authors	Mutation	Protein	Receptor	ASD	ID	Epilepsy (onset)	RTT	Hypotonia	Ataxia	Dystonia
EuroEPINOMICS 2014	c.2084G>T	p.Ser695Ile	TM6	-	yes, severe	yes (1.5m)	-	yes	-	-
EuroEPINOMICS 2014	c.2114T>A	p.Ile705Asn	TM6	yes	yes, severe	yes (2.5m)	yes	yes	yes	-
Lopes 2016	c.1699G>A	p.Ala567Thr	TM3	yes	yes, severe	no	yes	-	-	**yes (lower limbs)**
Hamdan 2017	c.2077G>T	p.Gly693Trp	TM6	-	yes, severe	yes (11m)	-	yes	-	-
DDD Cohort 2017	c.1699G>A	p.Ala567Thr	TM3	-	yes	-	-	-	-	-
DDD Cohort 2017	c.1181C>T	p.Thr394Met	N-Ter	-	yes	-	-	-	-	-
DDD Cohort 2017	c.1181C>T	p.Thr394Met	N-Ter	-	yes	-	-	-	-	-
Yoo 2017	c.1699G>A	p.Ala567Thr	TM3	-	yes	yes	yes	-	yes	-
Yoo 2017	c.1699G>A	p.Ala567Thr	TM3	-	yes	no	yes	-	-	-
Yoo 2017	c.1699G>A	p.Ala567Thr	TM3	yes	yes	no	-	yes	yes	**yes**
Yoo 2017	c.1699G>A	p.Ala567Thr	TM3	-	yes	no	-	-	-	-
Carneiro 2018	c.1699G>A	p.Ala707Thr	TM3	-	yes	-	-	-	-	-
Vuilaume 2018	-	p.Ala707Thr	TM6	-	yes, severe	no	yes	-	-	-
Takata 2018	c.1699G>A	p.Ala567Thr	TM3	yes	-	-	-	-	-	-
Chin 2019	del 9q22.33	-	-	-	-	yes	-	-	-	-
Samanta 2019	c.1318G>A	p.Gly440Arg	N-Ter	-	yes, mild	yes	-	-	-	-
Kim 2020	c.2002A>C	p.Met668Leu	TM5	-	-	yes	-	-	-	-

Legend: ASD Autism spectrum disorder, ID intellectual disability, RTT Rett-like syndrome, TM transmembrane, N-Ter N-terminal domain, m months, y years, - not reported