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Short communication

Novel variants and phenotypes widen the phenotypic spectrum of *GABRG2*-related disorders

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ABSTRACT

Purpose: Next-generation sequencing (NGS) has made genetic testing of patients with epileptic encephalopathies easier – novel variants are discovered and new phenotypes described. Variants in the same gene – even the same variant – can cause different types of epilepsy and neurodevelopmental disorders. Our aim was to identify the genetic causes of epileptic encephalopathies in paediatric patients with complex phenotypes.

Methods: NGS was carried out for three patients with epileptic encephalopathies. Detailed clinical features, brain magnetic resonance imaging and electroencephalography were analysed. We searched the Human Gene Mutation Database for the published *GABRG2* variants with clinical description of patients and composed a summary of the variants and their phenotypic features.

Results: We identified two novel *de novo* GABRG2 variants, p.P282T and p.S306F, with new phenotypes including neuroradiological evidence of neurodegeneration and epilepsy of infancy with migrating focal seizures (EIMFS). One patient carried previously reported p.P83S variant with autism spectrum disorder (ASD) phenotype that has not yet been described related to *GABRG2* disorders and a more severe epilepsy phenotype than reported earlier. In all, the literature search yielded twenty-two articles describing 27 different variants that were divided into two categories: those with self-limiting epilepsies and febrile seizures and those with more severe drug-resistant epileptic encephalopathies.

Conclusion: This study further expands the genotypic and phenotypic spectrum of epilepsies associated with *GABRG2* variants. More knowledge is still needed about the influence of the environment, genetic background and other epilepsy susceptibility genes on the phenotype of the specific *GABRG2* variants.

Abbreviations: ASD, autism spectrum disorder; HGMD, human gene mutation database; NGS, next-generation sequencing; EIMFS, epilepsy of infancy with migrating focal seizures

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1. Introduction

Epileptic encephalopathies are a group of severe, usually childhood-onset disorders with frequent epileptic seizures and developmental delay or regression [1]. When genetic testing is available, it is possible to make a genetic diagnosis for 10–50% of epileptic encephalopathies [2,3]. There are more than 265 genes involved in the pathogenesis of epilepsy identified so far, including genes coding $GABA_A$ receptor subunits [3]. $GABA_A$ receptors are the primary mediators of fast inhibitory synaptic transmission in the central nervous system (CNS). Receptors are formed by pentameric assemblies of different subunit subtypes, the majority containing two α subunits, two β subunits and a γ or δ subunit [4]. Subunit γ, expressed mainly in the brain, is encoded by the *GABRG2* gene (HUGO Gene Nomenclature Committee identifier: 4087), and it has different isoforms produced by alternative splicing [5].

Variants in the *GABRG2* gene are associated with a variety of seizures and epilepsy types from self-limiting febrile seizures to drug-resistant epilepsies with comorbidities. The penetrance and the phenotype of variants in the *GABRG2* can vary markedly even within the same family. Only recently a more severe epileptic encephalopathy phenotype was described in patients with highly penetrant *de novo* missense variants [4,6,7].

Here we describe two novel *de novo GABRG2* variants associated

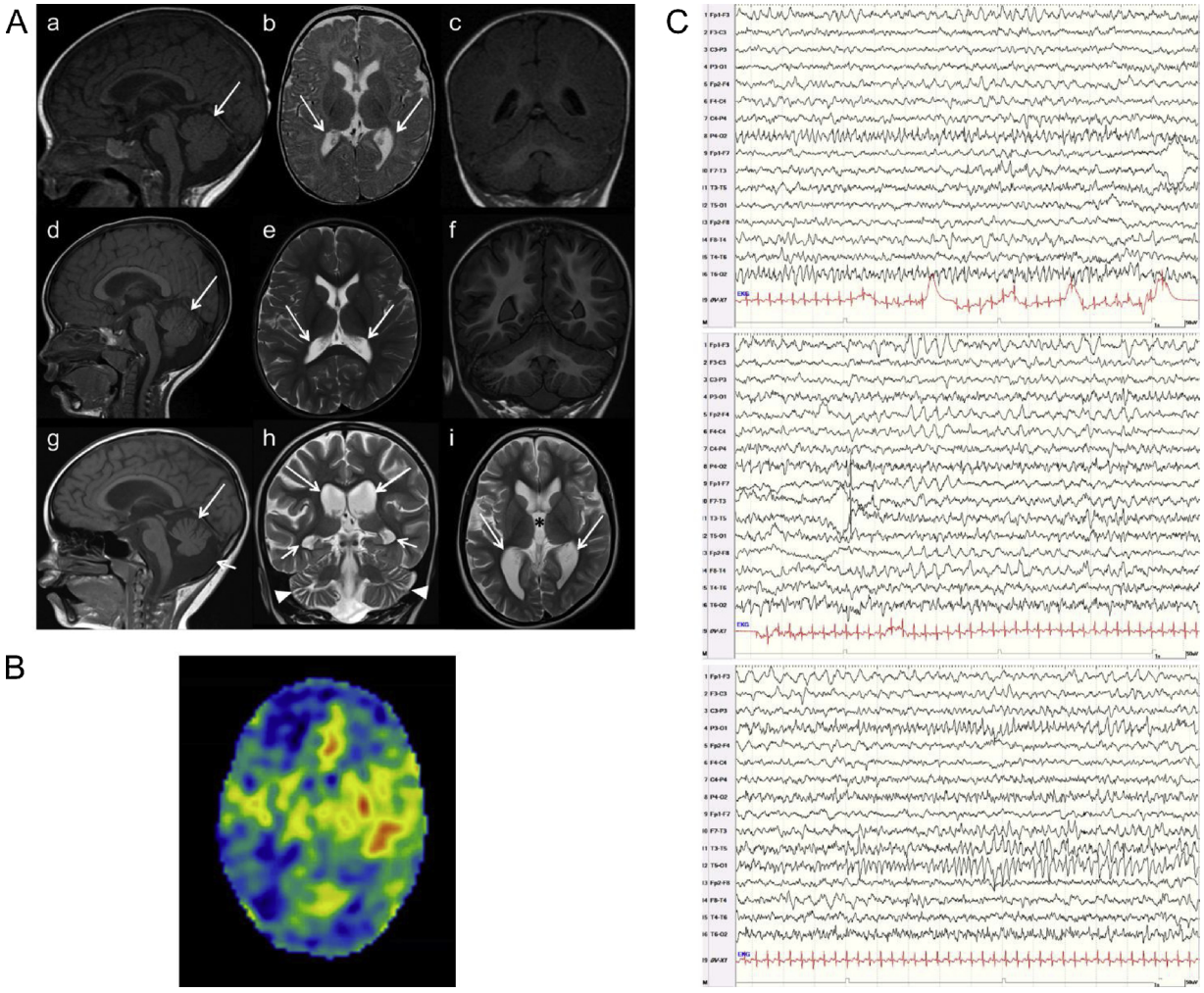


Fig. 1. Brain MRI findings of the patient 1 with the GABRG2 p.P282T variant (Aa-i). Arterial spin labelling (ASL) (B) and video EEG findings (C) of the patient 2 with the GABRG2 p.S306F variant.

A. T1-weighted (T1W) sagittal image (a) shows normal cerebellar vermis (long arrow) for patient 1 at the age of six months. T2-weighted (T2W) axial image (b) demonstrates slightly dilated lateral ventricles (long arrows). T2W axial (b) and T1W fluid attenuation inversion recovery (FLAIR) coronal (c) images show no dilatation of extra-axial cerebral or cerebellar cerebrospinal fluid (CSF) spaces.

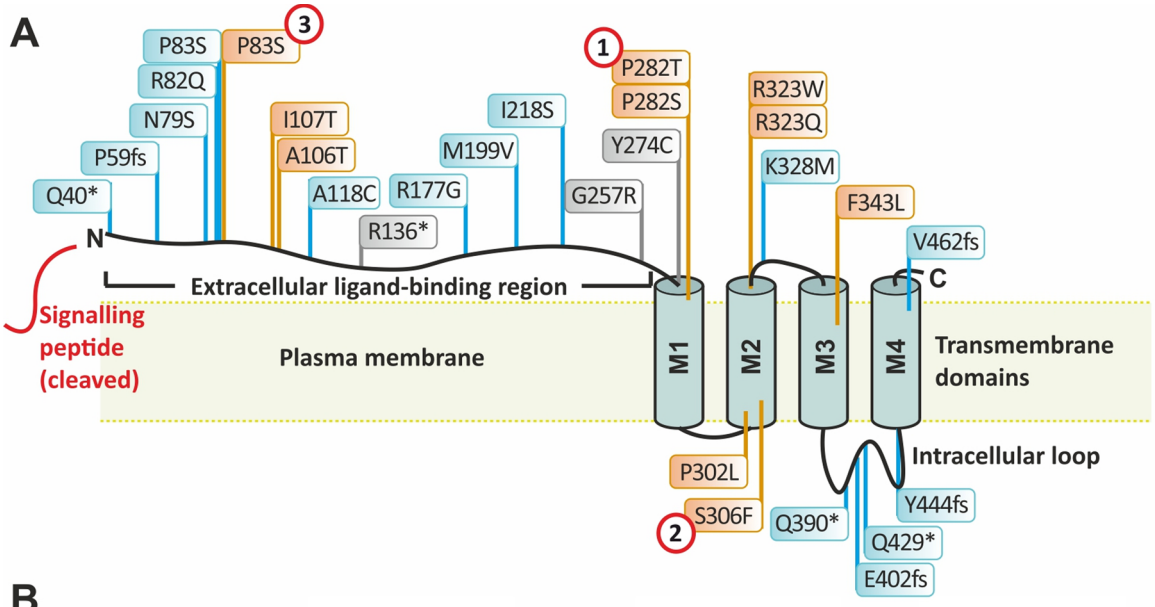
T1W sagittal image (d) shows normal cerebellar vermis (long arrow) at two years of age. There is no progression of the dilation of the lateral ventricles on axial T2W image (e, long arrows). The extra-axial CSF spaces are normal on T2W axial (e) and T1W FLAIR coronal (f) images.

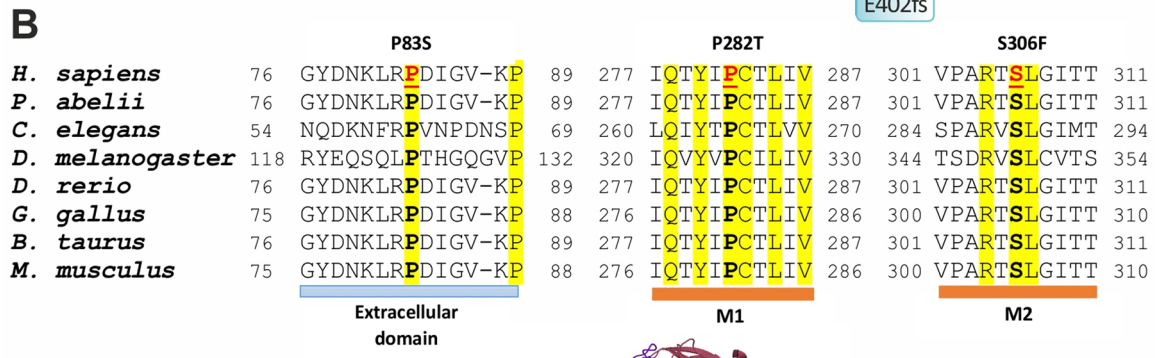
T1W sagittal image (g) demonstrates atrophic vermis (long arrow) and T2W coronal image (h) shows enlargement of the cerebellar CSF spaces (arrowheads) at nine years of age. Because the volume of the cerebellar lobes and vermis is decreased, the cisterna magna has been dilated (g, short arrow). T2W axial (i) image shows dilatation of the third ventricle (black star) and lateral ventricles (h and i, long arrows) and enlargement of the extra-axial CSF spaces revealing parenchymal loss due to cerebral atrophy. The hippocampal volume is decreased (h, short arrows).

B. ASL image through the rostral aspect of the lateral ventricles in patient 2 at seven weeks old demonstrating focal cerebral hyperperfusion in the left hemisphere, presumably due to ongoing seizure(s).

C. Video electroencephalogram (EEG) depicting a migrating focal seizure without associated clinical signs in patient 2 at 11 weeks old. In the first panel, the seizure is already underway in the right occipital (O2) region. It subsequently spreads to the left posterior head region (O1-T5) over the next 15 s. EEG calibration scale is included in the bottom right corner.

with new phenotypes and one patient with a previously published variant associated with a novel phenotype. We also searched the Human Gene Mutation Database (HGMD) for published *GABRG2* variants with clinical description of patients, and made a summary of the variants and their phenotypic features. This article widens the spectrum of *GABRG2*-related disorders to include a severe neurodegenerative disease phenotype and other severe, refractory epileptic encephalopathies.





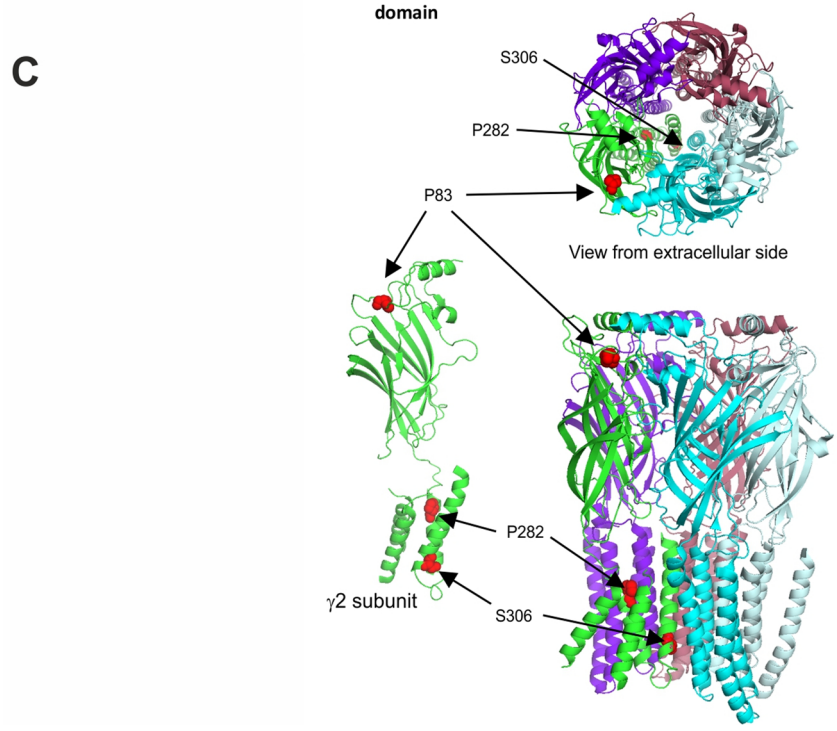


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Fig. 2. Genotype-phenotype comparison of the novel and the earlier published GABRG2 variants (A), conservation of the amino acid residues in different species (B), and 3D image of the mutation sites (C).
A. Schematic representation of the GABRG2 protein (UniProtKB – P18507) consisting of the signalling peptide, extracellular ligand-binding region, intracellular loop and four transmembrane domains (M1-4). The locations of previously published GABRG2 variants, as well as the ones described in this study (p.P282 T (1) and p.S306F (2) represent novel variants and p.P83S (3) a novel phenotype, in the protein are shown. The boxes, which represent the variants, have been coloured according to disease phenotypes as follows: light blue - mostly self-limiting seizures and/or epilepsy, not fully penetrant; orange - usually drug resistant and penetrant, epileptic encephalopathy; grey - no described phenotype. *GABRG2* variants and their phenotypes are listed in Suppl.Tbl.2.
B. Sequence alignment of GABRG2 homologs reveal that GABRG2 is highly conserved between different species. Human GABRG2 protein sequences around the mutation sites (*Homo sapiens*, Uniprot ID: P18507) were compared with protein sequences from Sumatran orangutan (*Pongo abelii*, Uniprot ID: Q5REA1), nematode (*Caenorhabditis elegans*, Uniprot ID: G5ECN1), fruit fly (*Drosophila melanogaster*, Uniprot ID: Q9VXL9), zebrafish (*Danio rerio*, Uniprot ID: F1RDP2), chicken (*Gallus gallus*, Uniprot ID: P21548), cattle (*Bos Taurus*, Uniprot ID: P22300) and house mouse (*Mus musculus*, Uniprot ID: P22723). The fully conserved residues between the studied species are highlighted in yellow. All three studied GABRG2 missense variants cause amino acid substitution in fully conserved residues (highlighted in red) and are predicted to be pathogenic.
C. Topology of the mutation sites P83, P282 and S306 of the γ2 subunit of the gamma- aminobutyric acid type A receptor. The 3D coordinate file 6D6T.PDB was used for visualization with PyMol (www.pymol.org) [13].

2. Patients

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration (1975, and revised in 2000). Written informed consents were obtained from the parents of the patients who were included in the study.

2.1. Case reports

a) Patient 1

Patient 1 is a 12-year-old girl who was referred for further investigations at the age of three months due to poor eye contact and hypotonia. She has drug-resistant Lennox-like epilepsy, profound developmental disability and dysmorphic features. Her disease course has been progressive. She developed pendular rotatory nystagmus and limb stiffness and her facial features became gradually coarse (video 1, Table 1, Suppl. Fig. 2). Due to a suspicion of sodium valproate-induced liver dysfunction, sodium valproate was replaced by zonisamide. Brain magnetic resonance imaging (MRI) findings are described in Fig. 1A.

Whole-exome sequencing detected a *de novo* pathogenic heterozygous missense variant in the *GABRG2* gene, c.844C > A (p.P282T) (Table 1, Fig. 2A–C). This variant was reported in one patient without clinical information in Clinical Variants database (ClinVar) but was not found in the general population (Suppl. 1, Suppl. 2) and was predicted to be pathogenic by Polyphen-2 (0.996) and SIFT (0).

b) Patient 2

Patient 2 is an 11-month-old boy with epilepsy of infancy with migrating focal seizures (EIMFS) (Table 1). Pregnancy and delivery were uncomplicated. Initial seizures began as an apnoea on the second day of life confirmed by the continuous video EEG. Current seizures consist of clonic arm jerking on one side or in both legs, occurring multiple times per day. A number of anti-seizure medications have failed to provide a sustained significant benefit (Suppl.1). A neurological examination reveals moderate generalized hypotonia, poor sucking and poor eye contact.

Multiple continuous video EEGs demonstrate frequent multifocal epileptiform discharges along with focal seizures that may spread to the contralateral hemisphere (Fig. 1C). A brain MRI at seven weeks old using arterial spin labelling (ASL) magnetic resonance perfusion imaging demonstrated left perisylvian hyperperfusion (Fig. 1B) but was otherwise normal.

An epilepsy panel comprising 83 genes associated with epilepsy revealed a *de novo* pathogenic heterozygous *GABRG2* c.917C > T (p.S306F) variant not found in the general population in publicly available databases (Suppl. 1, Suppl. 3, Table 1, Fig. 2A–C) and predicted to be pathogenic by Polyphen-2 (1.0) and SIFT (0.012).

c) Patient 3

Patient 3 is a seven-year-old boy with intractable Lennox-Gastaut syndrome and autism spectrum disorder (ASD), regressive type (Table 1). The pregnancy, delivery, and initial developmental course were normal. Language delay was evident at the age of one year. By the age of three years, with early intervention, he was speaking by using three-word phrases, singing and was completely toilet trained. However, he began to regress at the age of four years and he was diagnosed with ASD.

Seizures started as an unprovoked generalized tonic-clonic seizure at the age of nine months, lasting up 20 min, along with atypical absence seizures. A variety of anti-seizure medications have failed to offer significant benefit or caused significant side effects (Suppl. 1), except for clobazam, which was started most recently and has appeared to result in considerable improvement at a low dose of 0.2 mg/kg/day. A clinical neurological examination revealed very limited expression and comprehension and poor eye contact. Brain MRI was normal.

An epilepsy gene panel identified a heterozygous variant of uncertain significance (VUS) in *GABRG2* (c.247C > T, p.P83S) that was predicted to be pathogenic by Polyphen-2 (1.0) and SIFT (0.01) (Table 1, Fig. 2A–C). This particular variant has been reported in the literature (Table 1). [8] It is not observed in the general population in publicly available databases.

3. Results and discussion

In this study we describe two novel *de novo* GABRG2 variants, p.P282T and p.S306F, with new phenotypes including neuroradiological evidence of neurodegeneration and EIMFS and one patient with a previously published variant, p.P83S, with ASD not associated with GABRG2 variants so far.

The location of variants in different functional domains of the receptor has been found to correlate with the epileptic encephalopathy phenotype [6]. p.P282T variant is located in the transmembrane domain M1 of GABRG2 and it delineates the pore region of the receptor (Fig. 2C). A similar substitution, p.P282S, has been described recently, where pro-282 is substituted with serine [6]. Both threonine and serine are polar amino acids and very similar in their effects on protein structure. Therefore, one could expect that a p.P282T would have similar effects on the structure and function of the $GABA_A$ receptor as p.P282S. Indeed, patients with p.P282T and p.P282S have similar phenotypic features, except for the neurodegenerative disease course of our GABRG2 p.P282T patient.

Functional characterisation of the GABRG2 p.P282S mutant has revealed that the variant has an effect on the stability of the GABRG2 subunit, causing accumulation of the subunit in the endoplasmic reticulum (ER) and impairing the surface trafficking [6]. GABRG2

Table 1
Table of the variants and phenotypic features of the patients. Patients 1-3 are from this study. Phenotypes are compared with earlier published cases with p.P282S [6] and p.P82S [8] variants.

A

Individual	Patient 1	Patient 4 [6]	Patient 2	Patient 3	C-I-01 [8]	C-II-02 [8]	C-II-03 [8]	C-II-05 [8]	C-II-06 [8]	C-III-01 [8]	C-III-02 [8]	C-III-03 [8]	C-III-04 [8]	C-III-05 [8]
Variant	c.844C > A, p.P282T	c.844C > T, p.P282S	c.917C > T, p.S306F	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S	c.247C > T, p.P83S
Origin	*de novo*	*de novo*	*de novo*	paternal, VUS	NA	paternal	paternal	paternal	paternal	maternal	maternal	maternal	maternal	maternal
Seizure types	atonic, GTCS, eyelid myoclonia	SG, atypical absence	hemi clonic	GTCS, absence	–	febrile sz, single GTCS	febrile sz	febrile sz	febrile sz	–	febrile sz, absence	absence, GTCS	febrile sz	febrile sz
Epilepsy type or syndrome	Lennox-like	NA	EIMFS	Lennox-Gastaut syndrome	–	–	–	–	–	–	IGE	IGE	–	–
Age at epilepsy onset	4 -7 years	1 year	2 days	9 months	–	–	–	–	–	–	13 years	11 years	–	–
Drug resistant epilepsy	+	+	+	+	–	–	–	–	–	–	NA	NA	–	–
Developmental delay	+	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Autism spectrum disorder	–	NA	–	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Non-verbal/Non-ambulatory	+/+	+/NA	+/+	+/+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Nystagmus	+; rotatory; video 1.	roving eye movements	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dysmorphia	+; Suppl. Fig. 2	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Gastrostomy feeding	+	NA	+	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hypotonia	+	+	+	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Movement disorder[a]	+	NA	–	–	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EEG	GSW	GSW	Focal migrating, Fig. 1C.		NA	NA	NA	NA	NA	GSW	GSW	GSW	NA	NA
Brain MRI	Atrophy Fig. 1A.	Normal	Abnormal ASL; Fig. 1C.	Normal	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

- = not present; + = present; ALS = Arterial spin labelling; d = days; EIMFS = Epilepsy of infancy with migrating focal seizures; EMc = eyelid myoclonia; Fig = figure; GTCS = generalised tonic clonic seizures; GSW = generalised spike waves; HC = hemiclonic; mo = months; NA = not available; SG = secondarily generalised; sz = seizure; VUS = variant of uncertain significance; y = year.
[b]Movement disorders were as follows: Patient 1: generalised athetosis, stereotypical hand postures and movement, *e.g.* rocking, crossing hands in midline, hugging herself, pressing own eyes with hands, pulling own hair.

p.P282S variant causes disruption in structural domains leading to impaired function of $GABA_A$ receptor manifested by decreased GABA potency and slower deactivation in *in vitro* experiments [6].

Variant p.S306 F is located in the transmembrane domain M2 and it is near to the *de novo* missense variant p.P302L recently linked to Dravet syndrome [9]. Both p.P302L and p.S306 F are predicted to be common pore-lining residues that form part of the inner face of the cavity predicted for wild-type GABRG2 structure [9] (Fig. 2C). p.P302L variant was shown to cause loss of function in GABRG2 by altering the conduction pathway of the receptor during gating transitions among closed, open, and desensitized states, which led to enhanced neuronal excitability.

GABRG2 p.P83S variant has previously been described in a family with idiopathic generalized epilepsy and the variant was found to segregate with the seizure phenotype [8]. However, they did not find a difference between p.P83S mutant and wild type in terms of electrophysiological responses [8]. In contrast, later studies did show that p.P83S variant had reduced $\alpha1\beta2\gamma2$ receptor surface expression due to impaired assembly into pentamers, ER retention and subsequent degradation resulting in functional changes. p.P83S mutant receptor showed decreased whole cell current amplitudes and increased Zn2 + sensitivity. Inconsistent results were probably due to different experimental setups [10].

It hasn´t been yet possible to make genotype-phenotype correlations in the GABRG2 related disorders. However, it seems that especially pathogenic *de novo* missense variants in the transmembrane domains cause epileptic encephalopathy phenotype whereas familial truncating frameshift and nonsense variants in the intracellular loop and M4 transmembrane domain are more likely to cause a more self-limiting epilepsy phenotype. Variants in the extracellular ligand has to be individually evaluated since each area has it´s specific function, *e.g.* glycosylation site in I107T [6].

In conclusion, the phenotypic spectrum caused by pathogenic *GABRG2* variants has widened remarkably. However, it is still difficult to estimate the prognosis and choose the best treatment until there is better understanding of functional mechanisms related to the variants at the cellular level. Most efforts to address the impact of GABRG2 variants on cell functions have been limited to performing electrophysiological experiments on expression systems lacking the proteome present in a neuron, while studies performed on more native environments, such as rodent brain slices, have remained relatively scarce. Thus, more rigorous model systems for electrophysiological characterization of GABRG2 variants are called for. Induced human-derived pluripotent stem (iPS) cells combined with CRISPR/Cas9 genetic editing offer a potential model system to study the functional effects of GABRG2 variants in differentiated neurons [11,12]. Finally, more needs to be learned about other epilepsy susceptibility genes and their effects on phenotypes.

The authors have no conflict of interest

This study has been approved by local ethics committees. Parents of the patients have given their informed consent and written permission for the use of photographs and videos.

This article has not been published and is not under consideration for publishing elsewhere. All authors have approved the article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.seizure.2019.03.010.

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