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Defining the phenotypic spectrum of *SLC6A1* mutations

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Summary

Objective—Pathogenic *SLC6A1* variants were recently described in patients with myoclonic atonic epilepsy (MAE) and intellectual disability (ID). We set out to define the phenotypic spectrum in a larger cohort of *SCL6A1*-mutated patients.

Methods—We collected 24 *SLC6A1* probands and 6 affected family members. Four previously published cases were included for further electroclinical description. In total, we reviewed the electroclinical data of 34 subjects.

Results—Cognitive development was impaired in 33/34 (97%) subjects; 28/34 had mild to moderate ID, with language impairment being the most common feature. Epilepsy was diagnosed in 31/34 cases with mean onset at 3.7 years. Cognitive assessment before epilepsy onset was available in 24/31 subjects and was normal in 25% (6/24), and consistent with mild ID in 46% (11/24) or moderate ID in 17% (4/24). Two patients had speech delay only, and 1 had severe ID. After epilepsy onset, cognition deteriorated in 46% (11/24) of cases. The most common seizure types were absence, myoclonic, and atonic seizures. Sixteen cases fulfilled the diagnostic criteria for MAE. Seven further patients had different forms of generalized epilepsy and 2 had focal epilepsy. Twenty of 31 patients became seizure-free, with valproic acid being the most effective drug. There was no clear-cut correlation between seizure control and cognitive outcome. Electroencephalography (EEG) findings were available in 27/31 patients showing irregular bursts of diffuse 2.5–3.5 Hz spikes/polyspikes-and-slow waves in 25/31. Two patients developed an EEG pattern resembling electrical status epilepticus during sleep. Ataxia was observed in 7/34 cases. We describe 7 truncating and 18 missense variants, including 4 recurrent variants (Gly232Val, Ala288Val, Val342Met, and Gly362Arg).

Significance—Most patients carrying pathogenic *SLC6A1* variants have an MAE phenotype with language delay and mild/moderate ID before epilepsy onset. However, ID alone or associated with focal epilepsy can also be observed.

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WEB RESOURCES

The ExAC browser: http://exac.broadinstitute.org; SIFT: http://sift.jcvi.org; Poly-Phen2: http://genetics.bwh.harvard.edu/pph2/; MutationTaster: http://www.mutationtaster.org

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1 | INTRODUCTION

The voltage-dependent γ-aminobutyric acid (GABA) transporter 1 (GAT-1), encoded by *SLC6A1*, is one of the major GABA transporters of the human central nervous system.[1] GAT-1 is expressed primarily in the nerve terminals of GABAergic interneurons but can also be found in astrocytes, and has been shown to be crucial for the reuptake of GABA from the synapses and for its clearing from the extracellular space.[2] In addition to the neocortex, GAT-1 is found in abundance in the cerebellum.[3] GAT-1 knockout mice exhibit spontaneous spike-wave discharges (SWDs) and absence seizures.[4]

In 2014, Dikow et al.,[5] described 3 patients with intellectual disability (ID), epilepsy/electroencephalography (EEG) abnormalities, ataxia and stereotypic hand movements, and a microdeletion at 3p25.3, including the 2 GABA transporter genes, *SLC6A1* and *SLC6A11.* Recently, de novo *SLC6A1* variants discovered by large exome sequencing studies were reported in 3 patients with ID, myoclonic atonic (MA) seizures, delayed speech and delayed walking, autism spectrum disorders, and absence seizures, as well as in a patient with epileptic encephalopathy.[6–8] In 2015, we reported de novo inactivating variants in *SLC6A1* in up to 4% of patients with myoclonic atonic epilepsy (MAE), suggesting that pathogenic *SLC6A1* variants might be specific for MAE.[9] MAE is a syndrome characterized by the presence of myoclonic–atonic seizures, usually in an otherwise normal child, which may typically develop cognitive impairment after seizure onset (reviewed in Guerrini and Aicardi).[10] Extensive searches in other epileptic encephalopathy cohorts, such as epileptic spasms or Lennox-Gastaut syndrome, failed to detect pathogenic variants in this gene.

Here we aim to better characterize the clinical presentation and electroencephalographic features associated with pathogenic *SLC6A1* variants.

2 | MATERIAL AND METHODS

Patients with presumed pathogenic variants in *SLC6A1* were collected through an international collaboration between epilepsy genetics centers worldwide. The cohorts included for screening consisted primarily of patients with ID or childhood epilepsies including childhood encephalopathies, without a previously identified genetic cause. Probands and families, when available, underwent clinical, neurophysiological, and genetic examinations. All the available EEG data were collected and reviewed by a single epileptologist (EG). Seizures were classified according to the International League Against Epilepsy (ILAE) classification.[11] Atypical absences were defined as absence seizures with additional clinical features (eg, myoclonic jerks) and with an EEG pattern of diffuse spike-wave <3 c/s with a slower onset and offset compared to typical absence seizures.[12] Cognitive development was based mainly on the assessment of the psychomotor development by the referring physicians; only in a few cases was a formal neuropsychological testing performed.

For the diagnosis of MAE, we adopted the following diagnostic criteria: (1) usually normal development before epilepsy onset and absence of brain structural abnormalities; (2) onset of myoclonic, myoclonic–atonic, or atonic seizures between 7 months and 6 years of age; (3) EEG: 2–3 Hz generalized spike/polyspikes-and-slow waves; (4) diagnoses of benign myoclonic epilepsy of infancy (BMEI), severe myoclonic epilepsy of infancy (SMEI), and Lennox-Gastaut syndrome (LGS) have been excluded. (https://www.epilepsydiagnosis.org/syndrome/epilepsy-myoclonic-atonic-overview.html).

All probands and/or their parents or legal guardians signed an informed consent form. The study was approved by the local ethics committees.

Variants were classified as pathogenic if they were (1) nonsynonymous, splice-site altering, or truncating changes; (2) predicted damaging by one or more prediction software programs (PolyPhen-2 [http://genetics.bwh.harvard.edu/pph2/], SIFT [http://sift.jcvi.org], and MutationTaster [http://www.mutationtaster.org]); (3) not present in >60 000 of nonepileptic controls in the ExAC browser (the exome aggregation consortium browser, see Web Resources); and (4) were either de novo changes, inherited from an unaffected mosaic parent, or inherited from an affected parent. We used Sanger sequencing to confirm all variants and to perform segregation analysis.

3 | RESULTS

We identified 30 unpublished patients with a pathogenic *SCL6A1* variant (24 probands and 6 affected family members). Moreover, we included additional clinical information of 4 previously published cases. One (Patient 25) had only briefly been described previously and the 3 remaining cases were included for electroencephalographic characterization (Patients 5, 28, and 34) (Table 1). In total, we studied 34 *SLC6A1*-positive patients. Gender ratio was 19 female to 15 male patients.

3.1 | Genetics

We report on a cohort of 24 previously unpublished probands with pathogenic *SLC6A1* variants. Twenty variants occurred de novo in the affected proband, and 2 variants segregated in a dominant manner with the phenotype in the affected family (Figure 1). In 2/24 probands (Patients 12 and 24), segregation analysis could not be performed, and the inheritance mode remained unclear. However, both probands had missense variants identical to those found in other affected probands in this study. We found 18 missense variants, 2 splice-site variants, 1 frameshift variant, 3 nonsense variants, and 1 in-frame deletion. The 18 missense variants all affect highly conserved nucleotides and are absent from control databases (Figure 2). Overall, the variants suggest that loss of function of GAT-1 is the likely disease mechanism, as reported previously.

3.2 | Cognitive development

Data on cognitive performance before epilepsy onset were available in 24/31 patients with epilepsy. Cognition before epilepsy onset was normal in 5/24 (Patients 8, 15, 17, 26, and 30), mildly impaired in 12/24 (Patients 2, 5, 6, 11, 13, 14, 16, 22, 24, 28, and 21), and moderately impaired in 4/24 (Patients 20, 21, 23, and27); 2 Patients had speech delay (3 and

10) and 1 had severe ID (Patient 29),. After epilepsy onset, cognition deteriorated in 11 of them (Patients 3, 8, 10, 13, 15, 16, 22, 24, 26, 30, and 32).

All subjects but 1 (Patient 17) had cognitive impairment at the time of the inclusion in the study.

Sixteen of 34 patients had mild ID, 12 had moderate ID, 3 had severe ID, and 2 had learning disabilities. The most common feature was language delay, described in 16/34, whereas 4 probands presented mainly nonverbal deficits (Patients 1, 25, 27, and 29). There was no apparent correlation between seizure control and cognitive profile.

A large proportion of the 34 *SLC6A1* mutation carriers had behavioral problems, such as aggressive behavior/irritability (Patients 4, 10, 12, 14, 16, and 29), attention deficit disorders (Patients 10, 13, 15, 21, 30, and 31), stereotypies/automatisms (Patients 12, 20, 23, 27, and 29), and autistic features (Patients 4, 5, 12, 16, 20, 22, 26, and 27).

3.3 | Other clinical features

Neurological examination revealed mild ataxia (Patients 4, 7, 13, 22, and 31) or unsteady gait (Patients 23, 26, 27, and 28) in 9/34 patients, hypotonia in 3 cases (Patients 1, 4, and 16), and impairment of fine motor skills (Patients 21 and 32) and tremor (Patients 15 and 33) in 2 probands each. One patient each had chorea (4), verbal dyspraxia (21) and dyskinesia (31). No consistent dysmorphisms or malformations were present.

3.4 | Epilepsy phenotype

Thirty-one of 34 individuals had epilepsy, namely 27 of 28 probands (27 unpublished and 4 previously published cases) and 4 of 6 family members. Mean age at seizure onset was 3.7 years (range 0.5–7 years); 2 probands had epilepsy onset before the age of 1 year. Mean age at follow-up was 11.3 years (range 2–21 years).

Affected individuals presented with a variety of seizure types: absences not further specified (14/31), atypical absences (10/31), atonic seizures (14/31), and myoclonic or myoclonic–atonic seizures (12/31) were the most common. A few subjects (5/31) also had generalized tonic–clonic seizures (GTCS) (Table 1).

At the time of diagnosis, epilepsy syndromes were described as MAE in 16 patients and LGS in 1 patient (Patient 25), early onset absence epilepsy (EOAE) in its atypical form (Patient 1), eyelid myoclonia with absences (Patient 22), and childhood absence epilepsy (CAE) (Patients 17 and 33). Seven further patients presented with a generalized epilepsy difficult to classify because of inconsistencies of the electroclinical picture such as absence seizures (up to 100/day), atypical EEG pattern, or because further clinical details and EEG data were not available. Finally, 3 patients were diagnosed with focal epilepsy: 1 had temporal lobe epilepsy (Patient 24) and the other 2 had atypical childhood epilepsy with centrotemporal spikes (aBECTS) (Patients 6 and 28). The diagnosis of aBECTS was based on the description of focal motor seizures during sleep and on the presence of centrotemporal spikes on EEG, together with several atypical features (ID with autistic

features or mild neurological deficits, the epilepsy history, and the topography of the EEG abnormalities).

Twenty of 31 patients with epilepsy became seizure-free. Ten of them were treated with valproic acid (VPA), either as monotherapy or in combination with clonazepam (CLZ), lamotrigine (LTG), or levetiracetam (LEV). The remaining 10 subjects became seizure-free on LTG, ethosuximide (ESM), LEV, and LTG or CLZ, alone or in combination. Five further patients were treated with VPA, all achieving partial seizure control. Vagus nerve stimulation (VNS) was attempted in 1 patient with LGS (Patient 25) without benefit. None of the patients reported herein has tried a ketogenic diet, recently described as potentially effective for MAE.

3.5 | Neurophysiological and imaging characteristics

EEG data were available in 27/34 *SLC6A1*-positive patients, all with epilepsy (23 novel cases and 4 previously published), and were abnormal in all cases. A slowing of the background activity was observed in 11/28 subjects and bursts of irregular generalized 2.5–3.5 Hz spikes/polyspikes and slow waves with a gradual onset in the occipital regions were present in 25/28 patients (Figure 3). In some instances, these epileptic discharges correlated with staring and loss of consciousness with or without a myoclonic or atonic component, in different combinations, resulting in atypical absences, myoclonic–atonic seizures, atonic seizures/drop attacks, or myoclonic seizures. Two further probands had focal epileptiform abnormalities in the centrotemporal (Patient 12) or frontotemporal (Patient 23) regions (the latter occasionally presenting generalized spike and slow waves during the hyperpnea). Of interest, in Patient 12, epilepsy evolved presenting generalized spike and slow waves and atypical absences at the latest observations.

Two patients (6 and 28) initially presenting with an MAE-like phenotype and irregular generalized spikes and slow waves, transitioned to showing only focal epilepti-form abnormalities in the centro-parietotemporal regions at the time of the inclusion in this study (school age). In both cases, the epileptiform abnormalities were markedly enhanced by sleep, as it occurs in encephalopathy with status epilepticus during sleep (ESES). In 1 patient, the spike-wave index during non–rapid eye movement (NREM) sleep was 82% (Figure 4) and in the other, the EEG was reported as “almost continuous epileptic activity” during NREM sleep. In neither patient was this EEG pattern associated with cognitive/behavioral deterioration as seen in ESES.

Brain magnetic resonance imaging (MRI) was unremarkable in the majority of *SLC6A1* mutation carriers for whom data were available (14/19). Five cases had nonspecific MRI abnormalities, such as mild vermian hypoplasia or enlarged frontal spaces.

4 | DISCUSSION

In the present study, we describe the clinical features of 34 patients with pathogenic variants in *SLC6A1* (24 probands, 6 family members, as well as 4 cases previously published[8,9]) and the EEG features of 28 of them.

The identified variants include several truncating variants supporting loss of function of GAT-1 as a disease mechanism, as reported previously.[6] Within this study, we found 4 recurring missense variants (Gly232Val, Ala288-Met, Val342Met, and Gly362Arg), suggesting possible mutational "hot spots" in *SLC6A1.*

In contrast to the previously published cases with *SLC6A1* variants, this cohort displays a broader phenotypic spectrum sharing some common key features.

4.1 | Cognitive and behavioral deficits

The clinical hallmark of the *SCL6A1* variant carriers is cognitive impairment. Almost all subjects (33/34) presented certain degrees of cognitive deficits at the time of observation, consisting of mild-to-moderate ID in 82% of the cases (28/34). Speech difficulties were a common feature, as reported previously, present in 17/34 of patients, whereas pure nonverbal deficits were uncommon. In half of the cases (17/34), we also observed behavioral problems, namely, attention deficit, hyperactivity, aggressive behavior, hand stereotypies, and autistic features in different combinations. This observation is supported by the data in the *Scl6a1* mouse model.[13] Recently, a study found that selected *SLC6A1* variants may correlate with a higher risk of Attention-deficit/hyperactivity disorder (ADHD) in humans.[14]

Of interest, 3/34 *SLC6A1* variant carriers never had epileptic seizures, but manifested only learnings disabilities or mild ID with or without autistic features, which represent a previously undescribed phenotype associated with *SLC6A1.* Two of these individuals had relatives, carrying the same variant, with an MAE phenotype, suggesting variable expressivity possibly due to either genetic or environmental modifying factors, and indicating that genotype–phenotype correlations are not straightforward.

4.2 | Epilepsy

Thirty-one of 34 patients had epilepsy, with a mean age at onset of 3.7 years. The majority (24/30) of the patients displayed absences as the main seizure type, in agreement with studies in mice, that have shown an electroclinical phenotype consistent with atypical absence seizures.[4] We identified 16 subjects classified as MAE. Doose et al. stated that MAE usually begins in previously normally developing children.[15] However, he did not propose a "rigid" syndrome, but rather a condition with unifying features plus a recognized phenotypic variability attributed to a multifactorial background. In our cohort, the most common phenotype associated with *SLC6A1* could indeed be classified as MAE, associated in most of the patients with various degrees of ID before epilepsy onset, as also observed previously.[9]

Five patients were diagnosed as having a variety of other generalized epilepsy syndromes, including phenotypes resembling CAE (Patient 25) and EOAE (Patient 1), in both cases associated with mild-to-moderate ID. The phenotypes of CAE and EOAE have previously been observed in *SLC2A1*, *GABRB3*, and *CACNA1H*,[16–18] but we now extend the genetic etiology of these 2 epilepsy phenotypes to include *SLC6A1.* Finally, in 4 patients, diagnosed as "unclassified generalized," the inconsistencies between the clinical features and the EEG findings did not allow a syndromic classification.

Two patients presented with a phenotype resembling aBECTS, although with atypical electroclinical features (Patients 6 and 28), and 1 had temporal lobe epilepsy (Patient 24). This has not been described in any of the S*lc6a1* animal models or human *SLC6A1* variant carriers.[19] The subjects with aBECTS presented an extreme activation of the epileptiform abnormalities during NREM sleep resembling an ESES pattern. Surprisingly, both subjects developed aBECTS as an evolution of an MAE-like phenotype. A third subject presenting with aBECTS at epilepsy onset (Patient 12), evolved into a generalized epilepsy difficult to classify later, suggesting that the electroclinical features of *SLC6A1*-related epilepsy are variable and can change over time. Finally, 1 patient (25) was diagnosed with an epileptic encephalopathy of the LGS type.

Data about cognition before epilepsy onset were available in 24/31 patients. Only 5 had normal cognitive development, whereas 64% of the subjects (16/24) presented mild-to-moderate ID. After epilepsy onset, cognition deteriorated in 44% of cases (11/24) and did not improve after seizure control was achieved. Therefore, the contribution of epilepsy to the development of the cognitive impairment in patients with a pathogenic *SLC6A1* variant is uncertain.

4.3 | EEG data

Most patients exhibited a similar EEG pattern consisting of irregular, generalized 2.5–3.5 Hz spike/polyspikes-and-waves, providing a neurophysiological link to the animal studies. The clinical correlate of these EEG discharges could be an absence seizure, in most cases with atypical features, and with atonic and/or myoclonic components (16/28 cases). In our cohort, differences in the EEG pattern and in the associated clinical manifestations led to different phenotypical classification such as MAE, LGS, EOAE, or CAE. In 3 patients, the epileptiform abnormalities transitioned from generalized to strictly focal in the centro-parietotemporal regions (Patients 6 and 28) and vice versa (Patient 12) during the disease.

4.4 | Epilepsy treatment

The limited data thus far available suggest a good response to VPA, either as monotherapy or in combination with other antiepileptic drugs (AEDs). Ten of 15 patients treated with VPA became seizure-free, and the remaining 5 had a partial benefit. VPA is thought to have a positive effect on the GABA system (possibly increasing the GABA concentration in the human brain.[20]), which could be part of the explanation for the favorable response to this drug.

4.5 | Neurological signs

Electroclinical studies in mice have suggested that the tonic inhibition in the brain also leads to motor disturbances, such as ataxia, which correlates well with the observation that the GABA transporters are highly present in the cerebellum as well as in cortical neurons.[1] We found that 29% (9/31) of the patients had mild ataxia or unsteady gait. A few patients displayed other cerebellar signs such as mild hypotonia, tremor, and fine-motor impairment, in accordance with the hypothesis of a slight perturbation of motor control in animal models.[4] Overall, we can conclude that humans carrying pathogenic *SLC6A1* variants do not seem to display severe neurological symptoms.

These data combined with those of Carvill et al.[9] suggest that typically *SLC6A1*-positive patients have mild-to-moderate ID, with language delay being one of the major features, preceding the onset of an epilepsy with an MAE phenotype. Therefore, in children with ID with or without behavioral disturbances, developing polymorphic seizure types, including absences, myoclonic and atonic seizures, and rare or no GTCS, screening for *SLC6A1* should be undertaken. In addition, we describe cases never developing epilepsy. Because most patients described to date are children or young adults, longitudinal follow-up will be important to understand how seizures and cognition may change with age.

With this study, we define the phenotypic spectrum of clinical manifestations associated with *SLC6A1* variants to include different forms of generalized epilepsies, but also a few cases of focal epilepsies as well as cases with ID without epilepsy. We confirm that the predominant epilepsy phenotype is MAE with preexisting mild-to-moderate ID with or without behavioral symptoms. Further studies are needed to confirm the phenotypic spectrum and to investigate the functional consequences of pathogenic variants in *SLC6A1.*

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Key Points

- *SLC6A1* mutations cause a wider phenotype than solely MAE
- The *SLC6A1* phenotype consists of absence seizures and mild-to-moderate intellectual disability
- The common EEG pattern comprises irregular, high ample, and generalized spike-and-waves

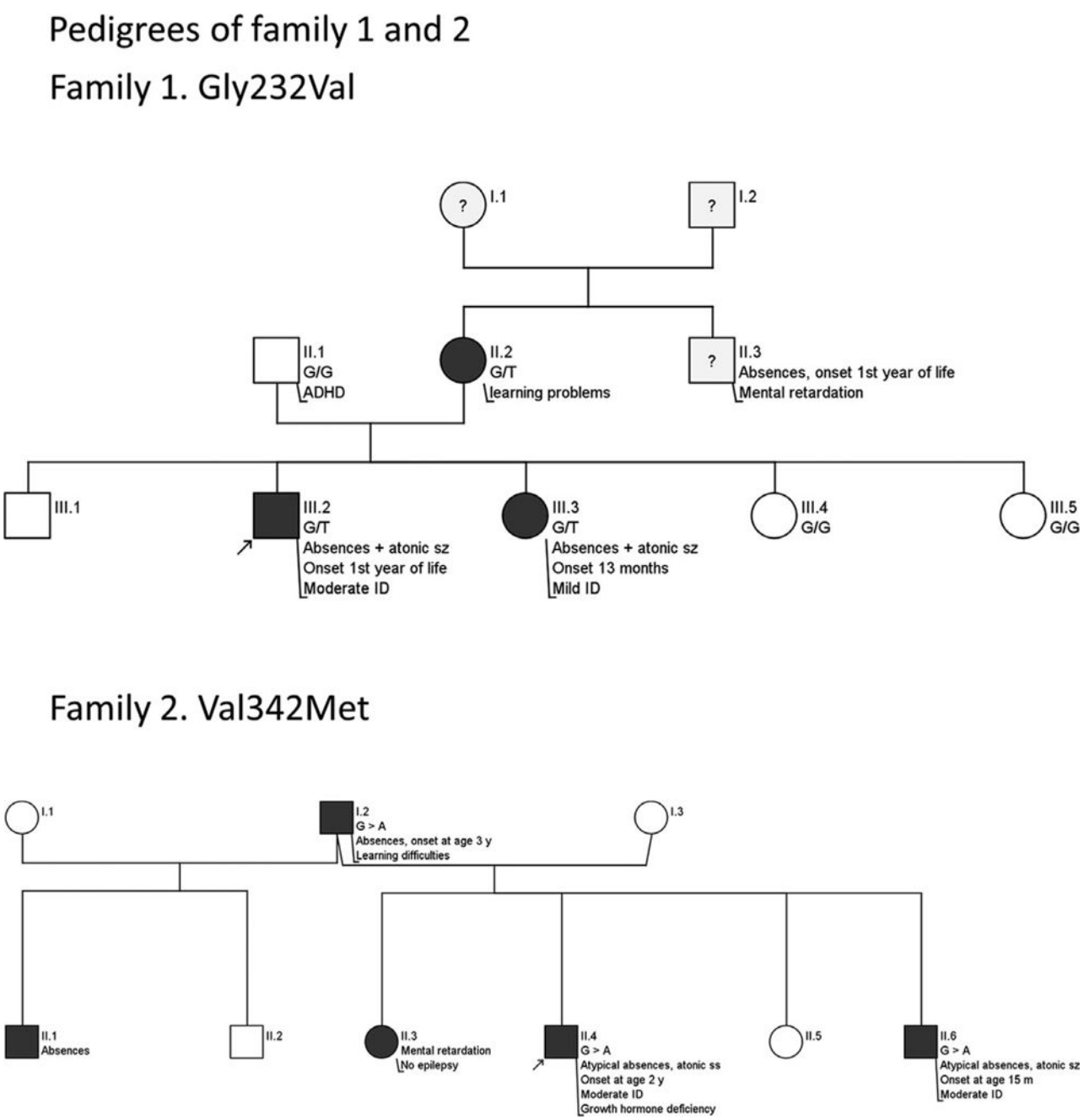

FIGURE 1.
Pedigrees of families 1 and 2

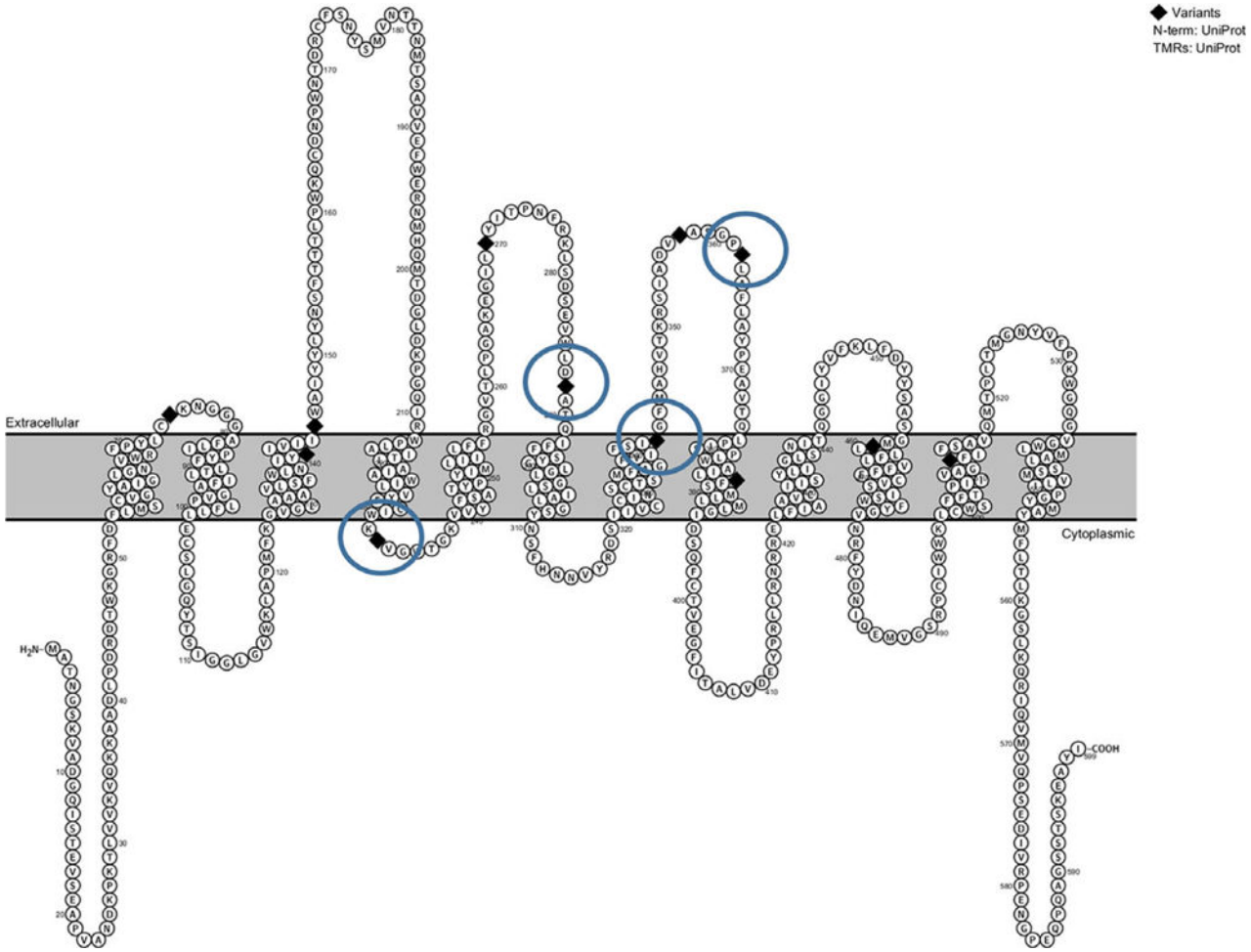

FIGURE 2.

The *SLC6A1* gene with the missense variants found in this study. Recurrent variants are highlighted

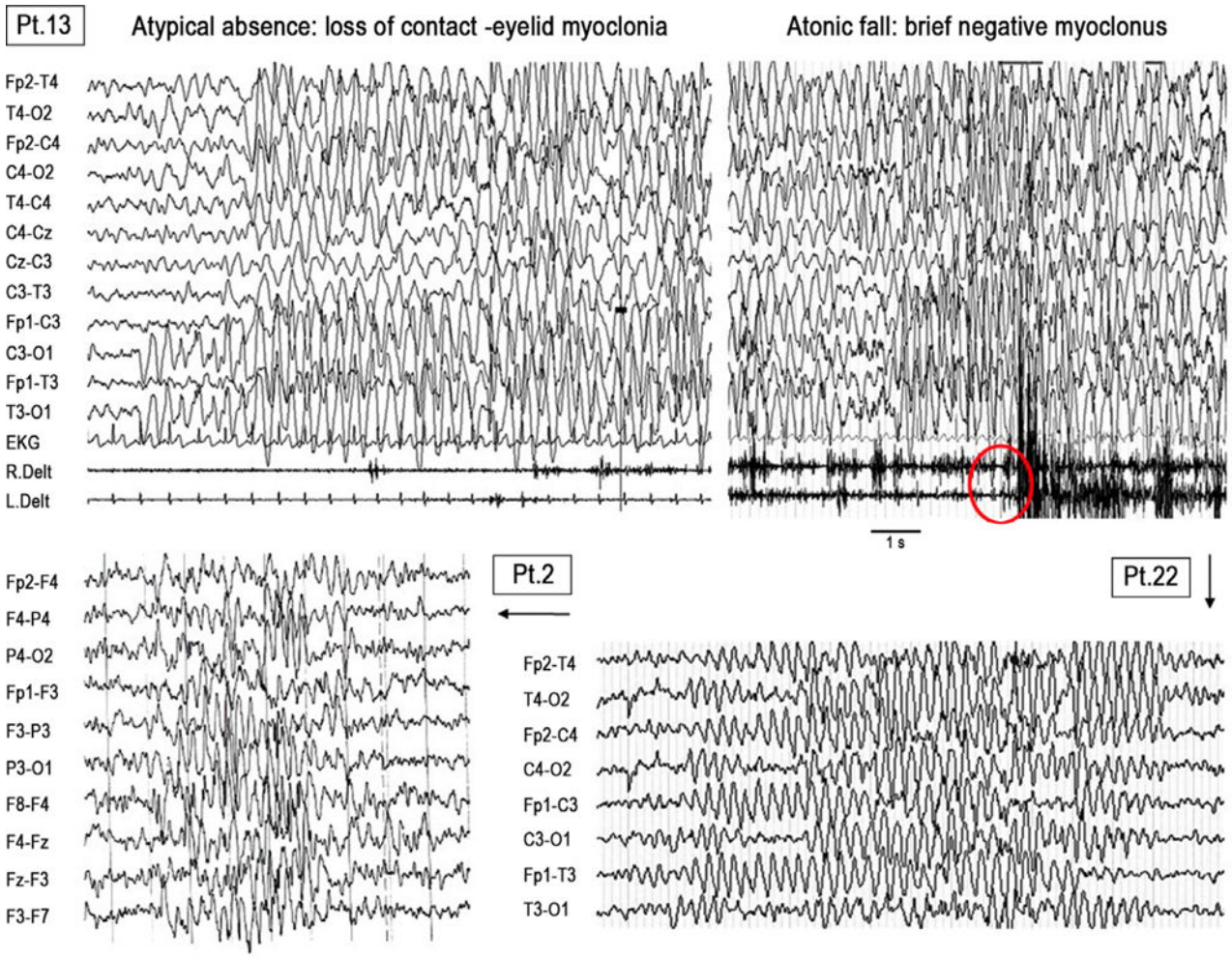

FIGURE 3.

Similar EEG pattern in 3 different patients diagnosed as MAE-like (Patient 20), unclassified generalized (Patient 2), and typical MAE (Patient 31). The EEG abnormalities consisted of prolonged bursts of irregular generalized 2.5–3.5 Hz slow waves with and without prominent spikes/polyspikes component, typically starting gradually in the occipital regions. In Patient 20, roughly the same EEG pattern can give rise to clinical manifestations consisting of atypical absence (to the left), or to a seizure with a brief atonic and myoclonic component (to the right). EEG parameters are the following. Patient 20: band pass filter 1–35 Hz, time constant 0.1 s, sensitivity 20 μV/mm; Patient 2: band pass filter 1–70 Hz time constant 0.3 s, sensitivity 10 μV/mm; Patient 31: band pass filter 1–30, time constant 0.16 s, sensitivity 10 μV/mm

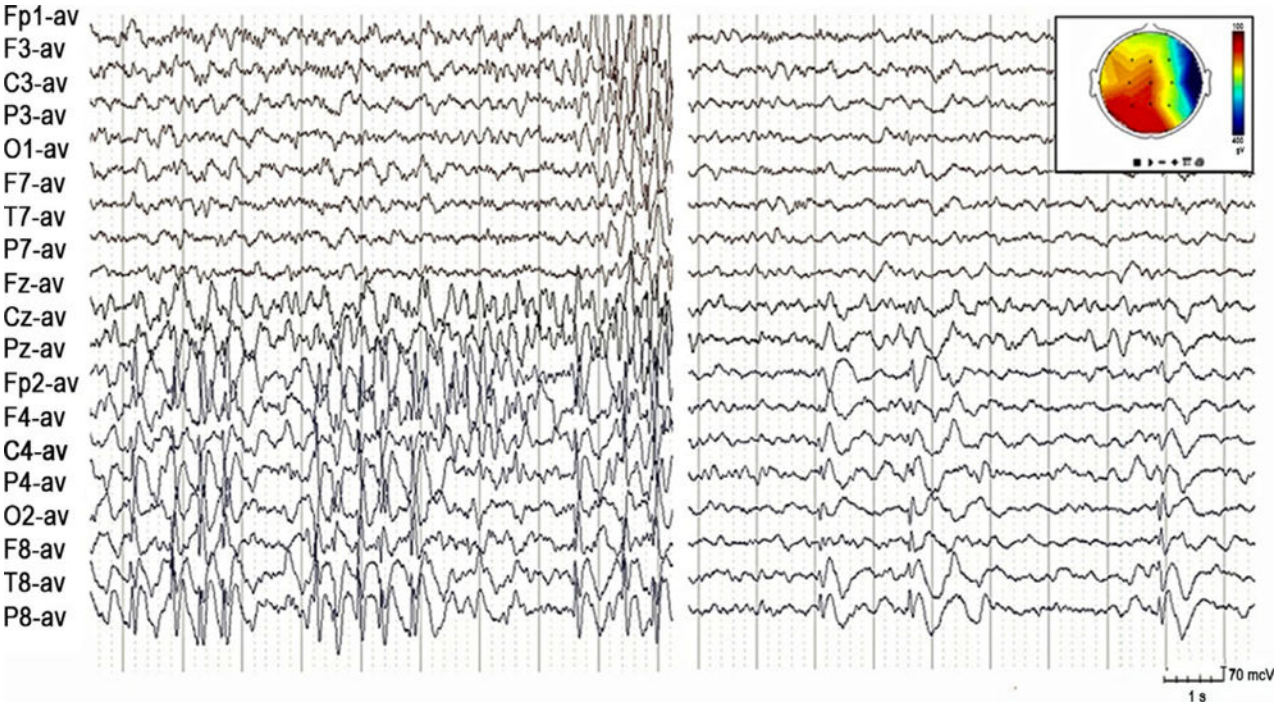

FIGURE 4.

Nocturnal sleep EEG in Patient 26 at the age of 4 y and 5 mo. During NREM sleep (to the left) the EEG is characterized by almost continuous spike and slow waves in the right hemisphere, with occasional spreading diffuse (spike–wave index 82%). During REM sleep (to the right) the epileptiform abnormalities are less frequent and more focal in the temporocentral region. This EEG picture strongly resembles hemispheric ESES, although the clinical correlate is vague. In the right upper corner, the amplitude map corresponding to the pick of the spike shows a radial orientation of the dipole. EEG parameters: band pass filter 1–70 Hz, time constant 0.1 s

TABLE 1

Clinical characteristics of *SLC6A1* variant carriers

Patient no.	Gender/ age at inclusion	Family history	Cognition before epilepsy onset	Age at epilepsy onset	Seizure type	EEG	Epilepsy syn-drome at diagnosis	Cognition after seizure onset	Behavioral problems	Neurological findings	Effective AED	Mutation
1	F/6 y	None	NA	5 mo	Atypical absences atonic, eyelid myoclonia	BG slowing bilat. occipital +/− generalized epileptic discharges	EOAE (atypical form)	Moderate ID	NA	Hypotonia	Sz free on VPA + CLZ	c.104dupA p.Lys36GluFsTer171 de novo
2	M/13 y	Father has spastic paraparesis (SP)	Mild ID	4 y	Absences (up to 100/day)	Generalized atypical SW	Unclassified generalized	Mild ID	None	Mild walking difficulties related to the SP inherited from his father	VPA (partially effective), sz free with VPA + LTG	c.223G>A p.Gly75Arg de novo
3	F/17 y	None	Speech delay	2 y	Myoclonic atonic, myoclonic, absences, nonconvulsive status	Generalized epileptiform activity	MAE	Mild to moderate ID	None	Normal	Sz free	c.419A>G p.Tyr140Cys de novo
4	F/28 mo	None	—	—	None	NA (referred as normal)	—	Mild ID	Autism, irritability	Mild hypotonia, ataxia, chorea	—	c.434C>T p.Ser145Phe de novo
5 Prev.publ. in Carvill et al.[9]	F/12 y	None	Mild ID	3 y, 2 mo	Absences, myoclonic atonic	BG slowing generalized SW (3 y, 6 mo) –> normal (4 y)	MAE-	Mild ID	Mild autistic traits	Normal	ESM, CBZ (partially effective), sz free with VPA	c.578G>A p.Thp193Ter de novo
6	M/10 y	None	Mild ID	2 y	Atypical absences (at onset)	Centrotemporal spikes (at evolution) Extreme activation during NREM sleep (7 y)	MAE evolving to aBECTS with an ESES-like pattern	Mild ID	None	Normal	NA	c.695G>T, p.Gly232Val de novo
7 Family 1, proband	M/17 y	Yes	NA	1 y	Absences, atonic	BG slowing Generalized SW (3 Hz)	MAE	Moderate ID	None	Mild ataxia	VPA, ESM, LTG (partially effective)	c.695G>T p.Gly232Val *matemal*
8 Family 1, brother	F/10 y	Yes	Normal	13 mo	Absences, atonic	BG slowing Generalized SW/poly-SW	MAE	Mild ID	None	Normal	Sz free with ESM	c.695G>T p.Gly232Val *matemal*
9 Family 1, mother	F/NA	NA	—	—	None	NA	—	Learning disabilities	None	NA	—	c.695G>T p.Gly232Val
10	M/7 y	None	Speech delay	18 mo	Atypical absences	Occipital notched rhythmic delta SW (2–2.5 Hz)	Unclassified generalized	Mild ID	ADHD, irritability	Normal	TPM, VPA (partially effective)	c.809T>C p.Phe270Ser de novo
11	M/8 y	None	Mild ID	22 mo	Absences, atonic, myoclonic	generalized SW (3 Hz)	MAE	Mild ID	None	Normal	Sz free with VPA, LTG	C.863C>T p.Ala288Val de novo
12	F/7 y	Psychiatric disease	NA	5 y	Focal (at onset), absences (at evolution)	Initially centrotemporal spikes → generalized SW	Initially BECTs-like → unclassified generalized	Moderate to severe ID	Autistic features, aggressive behaviors, stereotypies	Normal	LTG (partially effective)	C.863C>T p.Ala288Val *unknown*
13	M/12 y	None	Mild ID	18 mo	Atypical absences atonic, Myoclonic	BG slowing Generalized SW	MAE	Moderate ID	Attention deficit	Mild ataxia	Sz free with LTG + ESM	c.881_883del p.Phe294del de novo
14	F/11 y	None	Mild ID	5.5 y	Falls (seizure type not specified)	BG slowing Generalized slow waves and SW	MAE	Mild ID	Hyperkinetic, aggressive behaviors, excess, smiling	Normal	Sz free with VPA	C.987C>A p.Cys329Ter de novo
15 Family 2, proband	M/19 y	Yes	Normal	2 y	Atypical absences Atonic, GTCS	BG slowing Generalized SW Multifocal spikes	MAE	Severe ID	Attention deficit	Tremor	VPA + ESM (partially effective)	c.1024G>A p.Val342Met *patemal*
16 Family 2, brother	M/12 y	Yes	Mild ID	15 mo	Atypical absences, atonic, myoclonic atonic	BG slowing Generalized SW Multifocal spikes	MAE	Moderate ID	Autistic features, aggressive behaviors	Mild hypotonia	Sz free with ESM+ZSM	c.1024G>A p.Val342Met patemal
17 Family 2, brother	M/28 y	Yes-	Normal	childhood	Absences, GTCS	Generalized SW (3 Hz)	CAE	Normal	NA	Normal	VPA (partially effective)	c,1024G>A p.Val342Met *patemal*
18 Family 2, sister	F/NA	Yes	—	—	None	NA	—	Mild ID	None	Normal	—	c.1024G>A p.Val342Met *patemal*
19 Family 2, father	M/NA	None	NA	3 y	Absences	NA	Unclassified generalized	Learning disability	NA	NA	Treated with ESM till age of 17 y, now sz free	c,1024G>A p.Val342Met de novo
20	M/8 y	None	Moderate ID	5 y	Atypical absences	Generalized SW (3 Hz)	Unclassified generalized	Moderate ID	Rigidness, autism, stereotypies	NA	Sz free with VPA	c.1024G>A p.Val342Met de novo

Patient no.	Gender/ age at inclusion	Family history	Cognition before epilepsy onset	Age at epilepsy onset	Seizure type	EEG	Epilepsy syn-drome at diagnosis	Cognition after seizure onset	Behavioral problems	Neurological findings	Effective AED	Mutation
21	F/5 y	None	Moderate ID	2 y	Absences, atonic, myoclonic	Generalized SW and poly-SW (2.5–3.5 Hz)	MAE	Moderate ID	Mood swings, ADHD	Verbal dyspraxia, weak fine motor skills	NA	c,1024G>A p.Val342Met de novo
22	F/10	Autism, mother's side	Mild ID	13 mo	Eyelid myoclonia, absences	GSW and 3 Hz runs with occipital spikes; high voltage notched delta esp occipital.	Eyelid myoclonia with absences	Moderate ID	Autism spectrum	Ataxia	Sz free with VPA	c.l024G>A p.Val342Met de novo
23	F/9 y	None	Moderate ID	11 mo	Atypical absences, atonic	Generalized delta activity and SW SW left occipital Spikes/SW left centrotemporal	MAE	Mild ID	Hand stereotypies	Microcephaly Unsteady gait	Sz free with LEV + LTG	C.1070C>T p.Ala357Val de novo
24	F/21 y	NA	Mild ID	7 y	Focal, GTCS	Spikes left frontotemporal Generalized SW and generalized 3–4 Hz activity on HV	TLE	Moderate ID	None	NA	NA	c.1084g>a p.Gly362Arg unknown
25 Prev.publ. in Hal vors en et al.[8]	M/NA	None	NA	1 y	Atypical absences, Atonic, GTCS	Mild BG slowing 2 Hz generalized poly-SW	Lennox-Gastaut syndrome	Moderate ID	None	NA	None effective, VNS not effective	c.1084G>A p.Gly362Arg *from mosaic mother*
26	M/10 y	None	Normal	15 mo	Myoclonic atonic, myoclonic	3–4 second spike and wave complexes over posterior third of head provoked by eye closure and complete darkness	MAE	Mild ID	Autistic spectrum disorder	Broad based gait	Sz free with LEV	C.1155C>G p.Phe385Leu de novo
27	F/10 y	None	Moderate ID	3 y	Atonic, myoclonic, tonic/myoclonic	Generalized and lateralized (L or R) delta activity and SW	MAE	Moderate ID (nonverbal)	Autism, self-stim behavior, bruxism, rep. night waking	Brisk reflexes, unsteady gait (walked at 3.75 y)	Sz free with CLZ	c.1342A>T p.Lys448Ter de novo
28 Prev.publ. in Carvill et al.[9]	M/12 y	NA	Mild ID	3 y	Absences, atonic, myoclonic atonic → perioral myoclonia mainly during sleep, rare GTCS	Generalized poly-SW (at onset) Centrotemporal spikes (at evolution) Extreme activation during NREM sleep Now normal	MAE → aBECTs + ESES-like	Mild ID	None	Unsteady gait/balance problems	Sz free with LEV	c.1369_1370 delGG Gly457HisFsTer10 de novo
29	F/13 y	None	Severe ID	5 y	Absences	Generalized spikes/poly spikes	Unclassified generalized	Severe ID (almost nonverbal)	Aggressive behaviors, stereotypies	Normal (walked at 2 y)	Sz free with VPA	C.1377C>G p.Ser459Arg de novo
30	M/5 y	None	Normal	1 y	Atypical absences	BG slowing Bursts of irregular, diffuse spike and wave activity followed by diffuse delta Stereotyped focal spikes	Unclassified generalized	Mild ID (verbal)	ADHD	Normal	Sz improved with VPA/LTG	C.1531G>A p.Val511Met de novo
31	F/5 y	None	Mild ID	2 y	Atypical absences, atonic, myoclonic, eyelid myoclonia	BG slowing Bi occipital delta activity Generalized SW	MAE	Mild ID	ADD	Mild ataxia, dyskinesia	Sz free with ESM, LTG	C.1600C>T p.Gln534Ter de novo
32	F/4 y	NA	Normal	16 mo	Absences, atonic, myoclonic atonic	Generalized delta activity and SW (posterior prev.)	MAE	Mild ID	NA	Mild fine motor delay	Sz free with LEV + VPA	c.850-2A>G de novo
33	F/12 y	Sister with a 22ql3 deletion	NA	6 y	Absences	SW (3 Hz) Rare bifrontal spikes	CAE	Mild ID	NA	Action tremor	Sz free with VPA	c.6,1528-1G>C de novo
34 Prev.publ. in Carvill et al.[9]	F/7 y	None	NA	3 y	Absences, myoclonic atonic	BG slowing Generalized delta activity and SW	MAE	Mild/moderate ID	None	Normal	Sz free with VPA, LEV	3p25.3 del. including *SLC6A11* and *SLC6A1* (exon 1) de novo

Prev.publ., previously published; aBECTs, atypical benign childhood epilepsy with centrotemporal spikes; Ab, absence seizures; A, atonic seizures; AA, atypical absence seizures; ADD, attention deficit disorder; ADHD, attention-deficit/hyperactivity disorder; BG, background; CAE, childhood absence epilepsy; CLZ, clonazepam; ESES, electrical status epilepticus during sleep; rep., repeated; ESM, ethosuximide; EOAE, early onset absence epilepsy; F, female; GTC, generalized tonic–clonic; ID, intellectual disability; LEV, levetiracetam; LTG, lamotrigine; MAE, myoclonic atonic epilepsy; M, Male; m, months; NA, not available; NREM, non–rapid eye movement; Sz, seizure; SW, spike wave; TPM, topiramate; VPA, valproic acid; VNS, vagus nerve stimulation; y, years; ZSM, zonisamide; del., deletion.