European Journal of Paediatric Neurology 54 (2025) 121–129

Contents lists available at ScienceDirect

European Journal of Paediatric Neurology

journal homepage: www.journals.elsevier.com/european-journal-of-paediatric-neurology

Original article

Behavioral, neurodevelopmental profile, and epilepsy trajectory in two series of SLC6A1-NDD: A retrospective study with comprehensive assessment, and a participatory database study

Sarah Baer [a,b,*,1], Mathieu Rebert [a,d,1], Pauline Burger [b], Jean-Louis Mandel [b,c], Nathalie Villeneuve [e], Marc Gibaud [f], Cecilia Altuzarra [g], Frédéric Villega [h,i], Claude Cances [j], Laure Lacan [k,l], Sylvie Nguyen [k,l], Gaëtan Lesca [m,n], Hervé Isnard [o], Nouha Allani-Essid [p], Vincent Laugel [a,q], Romain Coutelle [r,s,t], Anne de Saint Martin [a,b]

[a] Department of Pediatric Neurology, Strasbourg University Hospital, Hautepierre Hospital, Centre de Référence des Épilepsies Rares, Strasbourg, France
[b] Institut de Génétique et de Biologie Moléculaire et Cellulaire, Université de Strasbourg, INSERM U1258, CNRS UMR7104, Illkirch, 67400, France
[c] University of Strasbourg Institute for Advanced Studies (USIAS), Strasbourg, France
[d] Physical Medicine and Rehabilitation Department, Clemenceau Institute and Strasbourg University Hospital, Strasbourg University, Strasbourg, France
[e] AP-HM, Hôpital Timone Enfants, Service de Neurologie Pédiatrique, Marseille, France
[f] Department of Pediatrics and Pediatric Emergency, Nantes University Hospital, Nantes, France
[g] Pediatrics Department, Besancon University Hospital, Besancon, France
[h] Université de Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, UMR, 5297, Bordeaux F-33000, France
[i] Department of Pediatric Neurology, CIC-0005, University Children's Hospital of Bordeaux, Bordeaux, France
[j] Department of Pediatric Neurology, Purpan University Hospital, Toulouse, France
[k] Service de neuropédiatrie CHU Lille, Lille, France
[l] ULR2694-METRICS, University of Lille, Lille, France
[m] Hospices Civils de Lyon, Service de Génétique, Centre de Référence des Épilepsies Rares, Lyon, France
[n] Institut Neuromyogène, Laboratoire Physiopathologie et Génétique du Neurone et du Muscle, Équipe Métabolisme énergétique et développement neuronal, CNRS UMR 5310, INSERM, U1217, Université Lyon 1, Lyon, France
[o] Pediatric Neurologist, Medical Office 28 rue de la république, Lyon, 69002, France
[p] APHP, Raymond-Poincaré Hospital, Department of Neuropediatrics, Paris, France
[q] Laboratoire de Génétique médicale, INSERM U1112, Institut de génétique médicale d'Alsace, Faculté de Médecine de Strasbourg, Hôpitaux Universitaires de Strasbourg, Strasbourg, France
[r] Hôpitaux Universitaires de Strasbourg, Service de psychiatrie de l'enfant et de l'adolescent, 1 place de l'hôpital, Strasbourg, 67000, France
[s] Centre d'excellence STRAS&ND, Strasbourg, France
[t] GIS Autisme et TND, Strasbourgs Teams, Strasbourg, France

ARTICLE INFO

Keywords:
SLC6A1
Developmental encephalopathy
Epilepsy
SLC6A1-NDD
Neurodevelopmental disorder

ABSTRACT

SLC6A1 (Solute Carrier Family 6 Member 1) variants are associated with *SLC6A1*-neurodevelopmental disorders (SLC6A1-NDD), which manifest as early-onset epilepsy, intellectual developmental disorder, and autism spectrum disorder. There have been over 300 reported cases so far. A retrospective analysis of 14 patients with *de novo SLC6A1* variants was conducted to assess their developmental milestones, epilepsy progression, antiseizure medication, and, for some, a comprehensive neurodevelopmental evaluation. Data from 14 additional families were also collected using the GenIDA participatory database, aiming to better characterize the natural history of genetic forms of NDDs.

Most patients exhibited normal early motor development, but delays in communication and language skills were observed. Their intellectual functioning varied, mostly falling within the low average to moderate intellectual developmental disorder range, with a predominant expressive and receptive language disorder. More than half of the group displayed autistic features, particularly stereotypic behavior. Behavioral disorders such as hyperactivity, anxiety, impulsivity, or inhibition were common concerns for parents.

* Corresponding author. Service de Pédiatrie 1, 1 avenue Molière, Cedex, 67082, STRASBOURG, France.
E-mail address: sarah.baer@chru-strasbourg.fr (S. Baer).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejpn.2025.01.003
Received 24 March 2024; Received in revised form 2 January 2025; Accepted 7 January 2025
Available online 10 January 2025

The first seizures occurred between 14 months and 5 years, mainly presenting as generalized seizures (atonic falls, absences, atypical absences, myoclonic-atonic seizures). EEG results frequently showed bursts of rhythmic delta activity, persisting from childhood to adulthood, with epilepsy primarily responding well to antiseizure medication in most of the reported cases.

This study exhibited a distinct electroclinical and neurodevelopmental phenotype in young children, suggesting the importance of early genetic testing for SLC6A1-NDD diagnosis.

1. Introduction

SLC6A1 (MIM*137165, *solute carrier family 6 member 1)* also known as GABA Transporter Type 1 is implicated in the reuptake of GABA from the synaptic cleft playing a key role in excitatory/inhibitory balance and GABA recycling [1]. On the molecular part, variants in *SLC6A1* result in protein folding and trafficking impairment leading to clearance of GABA from the synapse [2].

The loss of function of this protein-coding gene located on chromosome 3 (3p25.3) has been associated with epilepsy and neurodevelopmental symptoms. This discovery was made in 2015 in a cohort of patients with myoclonic atonic epilepsy [3]. With the improvement of genetic diagnosis, *SLC6A1* has been ranked in the top 10–20 genes [4] causing monogenic epilepsy. It has also been associated with other specific phenotypes such as Autism Spectrum Disorder (ASD) [5], Attention Deficit Hyperactivity Disorder (ADHD) [6], and schizophrenia [7]. The last clinically reported incidence is now 1/38,000 births worldwide [8].

The *SLC6A1*-neurodevelopmental disorder (NDD) can be characterized by mild to severe developmental delay and/or intellectual developmental disorder (IDD), generalized hypotonia of infancy, epilepsy with typical or atypical absence seizures, myoclonic-atonic seizures, or generalized tonic-clonic seizures. Patients are also described with ASD, ADHD, anxiety, or sleep disturbances. More than 300 patients [9] have been reported in the literature with a suggestive phenotype of specific NDD and have been studied as a whole cohort looking for clinical characterization, impact on caregivers [10], or Vineland tests [11].

The genotype-phenotype correlation is still debated in *SLC6A1*-NDD [9,12]. Recent publications have reported the variability of the phenotype especially in families with inherited variants [13], hypothesizing the existence of other genetic or environmental factors. Recent studies have shown a correlation between the variant and the level of GABA uptake. This correlation relates to the level of uptake and the pathogenic variants but does not indicate any correlation between the level of uptake and the level of IDD or epilepsy [14]. More recently a phenotype with developmental regression has been described that could be concordant with the starting of epilepsy [15].

This descriptive study may help the clinician screen and signal early problems, as reported by parents and standardized outcome measures, and introduce early rehabilitation and symptomatic treatments. With the emergence of potential therapeutic perspectives in this disorder, it seemed also important to us to better characterize the neurodevelopmental and behavioral profiles, as well as epilepsy, of these children, to have more accurate outcome measures. The purpose of the study was to describe the epilepsy characteristics, the neurodevelopmental and behavioral profiles of a multicenter French cohort. We then cross-checked our collected data with an international online participatory database (GenIDA) about *SLC6A1* patients.

2. Material and methods

2.1. Patients from the retrospective and multicomponent assessment study

We collected clinical, electrophysiological, therapeutic, and molecular data from 14 French-speaking patients affected with *SLC6A1* variants and their dedicated health care, through a national collaboration with the French Reference Center for Rare Epilepsies (CReER) network and geneticists' network (AnDDI-rare national health network). The advocacy group, *SLC6A1* Connect, especially, French-speaking families, were also informed about the study. Parents and patients were first contacted by email. Written informed consent and permission were obtained by the parents or legal guardian. This and all other study procedures complied with the Declaration of Helsinki and French legislation and regulations.

2.1.1. Data collected

Standardized clinical forms were filled out by the parents and divided into 3 parts: 1. General information (gender, age at the inclusion, global milestone achievement, personal and family background) 2. Global developmental trajectory especially concerning epilepsy, neurodevelopmental and behavioral aspects, previous testing results, education needs, and rehabilitation 3. Genetic results and additional tests. Physicians were also contacted to evaluate the progression of their *SLC6A1* patients.

All the number data are detailed considering median and standard deviation.

Characteristics of seizure were described according to the ILAE glossary [16].

At the molecular level, we considered variants as pathogenic based on a combination of the following criteria as suggested by Richards et al. [17] We used the *SLC6A1* reference transcript NM_003042.4. All variants have been found using targeted sequencing gene panel or whole exome sequencing confirmed using Sanger sequencing.

2.1.2. Neurodevelopmental and behavioral assessment

Some patients in the cohort had already been evaluated and we could collect the reports. For the others, we used 6 different neurodevelopmental, and behavioral scales: Vineland Adaptive Behavior Scale II [18,19], Autism Diagnosis Interview-Revised (ADI-R) [20], short sensory profile [21], Social Communication Questionnaire (SCQ) [22], Conners' Parent Rating Scale (CPRS) [23] and Aberrant Behavior Checklist (ABC) [24], which were administered during telephone interviews by a trained research assistant during 2020 and 2021.

A. Vineland Adaptive Behavior scale [18,19].

We used the French-validated version of VABS-II described in 2015, to assess adaptive behavior in individuals divided into three domains: communication, daily living skills, and socialization. For each subtest, we could define a developmental age and adaptative level. The adaptative level is defined according to standard scores (low between 20 and 70, moderately low between 71 and 85, adequate between 86 and 114).

B. Autism Diagnostic Interview-Revised [20].

The ADI-R is a standardized semi-structured interview used for the diagnosis of ASD (in verbal and nonverbal individuals), based on DSM-5 criteria. Three domains are explored: reciprocal social interactions (domain A), qualitative abnormalities in communication (domain B), and restricted, repetitive, and stereotyped behavior patterns (domain C). Each item can be scored from 0 to 3 with 0 meaning the absence of behavior and 3 when the abnormality was present.

The ADI-R supports a diagnosis of ASD when scores in all three domains (A, B, and C) are above cut-off scores. We used the French

validation of the ADI-R Social Communication Questionnaire.

C. Social Communication Questionnaire [22].

The severity of ASD symptoms was assessed using the Social Communication Questionnaire (SCQ) in French. This questionnaire is based on the ADI-R questions with 40 items answered by yes (=1) or no (=0).

D Short Sensory Profile [21].

The Short Sensory Profile is a 38-item questionnaire exploring seven sensory domains: tactile sensitivity, taste/smell sensitivity, movement sensitivity, under responsiveness/seeks sensation, auditory filtering, low energy/weakness, and visual/auditory sensitivity. All items are scored from 1 (always) to 5 (never). Based on the scores in each domain and the final score, the level of sensory impairment in the individual is categorized as typical performance, probable difference, or definite difference. This test has been validated in cohorts of children with IDD [25,26].

E. Aberrant Behavior Checklist [24].

Non-ASD behavioral problems were studied using the Aberrant Behavior Checklist (ABC), a 58-item rating scale used to identify the presence of maladaptive behavior in five categories: irritability; lethargy/social withdrawal; stereotypy; hyperactivity/noncompliance, and inappropriate speech. Each item is scored from 0 (not a problem at all) to 3 (a very significant problem), with a higher score indicating a more severe problem. This scale was designed for people with developmental disabilities and has previously been used in people with other genetic syndromes [27].

F. Conners' Parent Rating Scale

We used the 48-item Conners' Parent Rating Scale [23] (CPRS) to assess symptoms of inattention, distractibility, impulsivity, and hyperactivity. An ADHD index score is calculated based on responses to questions 7, 11, 13, 14, 25, 31, 33, 37, and 38. A score exceeding 15 suggests a likelihood of ADHD.

2.1.3. Participatory database study from GenIDA

We also collected data through GenIDA (Genetic of Intellectual Disability and Autism Spectrum Disorders: https://genida.unistra.fr) [28]. This international online participatory database aims to better characterize the clinical manifestations and natural histories of rare diseases using patient-reported outcome measurements (PROMs). We identified 17 SLC6A1-NDD patients. Three patients appeared in both cohorts because their parents also filled out the online database. We chose to include them only in the retrospective and multicomponent assessment study for more clarity. As a result, 14 patients from the GenIDA cohort have been included in the second part of the study. Clinical information is reported by parents of the GenIDA patients included in the study using a structured questionnaire exploring physical parameters, cognitive and behavioral aspects, the presence or absence of neurological disorders or problems affecting major physiological functions, as well as autonomy and quality of life. The closed questions are standardized for all genetic syndromes in this database. These patients couldn't be compared to the other reported patients in all the reported categories since we only collected data from a database with general questions, we reviewed the GenIDA PROMs in August 2024.

3. Results

Fourteen patients with *SLC6A1*-NDD patients (6 females and 8 males) have been included in the retrospective study with an age ranging from 3.5 to 45 years at the time of inclusion (median age 12 ± 10.9). None of them had remarkable birth or neonatal complications except patient 7, born at 30 weeks of gestation (WG) and patient 13, born at 33WG. All of them are French patients and have never been reported in the literature.

In the second part, we also described 14 other patients from the GenIDA international participatory database presenting with SLC6A1-NDD phenotype with an age ranging from 1,3 to 20,5 years (median age 5y ± 5,15) including 5 females and 9 males.

3.1. Cohort 1: 14 French patients' retrospective study with a comprehensive assessment

A Milestones achievement: a mild to severe language and communication delay

10 patients were considered without motor delay, and 3 patients started walking after 18 months. Patients started to walk at a median age of 18 months (± 1.96) but the age at first word was delayed with a median age of 2 years (± 2.36). The toilet training age was also reached later than the general population (Fig. 1 and Table S1) with a mean age of 5 years.

Language delay could be defined as the first word after 18 months, in our cohort 12/13 patients presented with the first word after 18 months and 1 developed earlier with the first words at 16 months.

Regarding education, 8 patients with learning disabilities were in school mainstreaming class, 3 needed a special school assistant, and 4 attended not-inclusive, special needs education. The oldest patient, now aged 45 years, had worked in an employment assistance centre for many years.

B Epilepsy characteristics

Seizures started at a median age of 2.3 years (± 1.3) from 14 months to 5 years. Most reported seizures were atonic falls (86 %), impaired awareness (79 %), myoclonic absences, and gestural automatisms. As previously reported tonic-clonic seizures were rare and involved only 35 % of the patients (n = 5), and no status epilepticus were reported. No typical febrile seizure was reported, but fever could induce an exacerbation of myoclonus. Besides the epileptic symptoms, paroxysmal episodes of ataxia could be noticed in the first years of life and some patients reported trembling (n = 5).

The EEG evidenced interictal anomalies with bursts of intermittent rhythmic delta activity that could be generalized and/or focal, with variable location, during wake or sleep state. The architecture of sleep is not always impacted. These anomalies could be persistent, with variable intensity through childhood, even if seizures had disappeared at the time of adolescence (Fig. 3).

All patients needed antiseizure medication (ASM) and 9 of them needed polytherapy. Various ASM or alternative therapies have been proposed: Levetiracetam, Valproate, Ethosuximide, Lamotrigine, Clonazepam, Clobazam, Topiramate, Vigabatrin, Steroids, ketogenic diet, or Vagus Nerve Stimulation. Ethosuximide (n = 10), Lamotrigine (n = 5), Sodium Valproate (n = 5), and Levetiracetam (n = 3) seem to be effective drugs concerning the progression of clinical seizure even if EEG could still show abnormalities. Vigabatrin or Topiramate seems to be aggravating with increasing seizures and behavioral disorders. Most of the patients benefited from 1 or 2 ASM at the same time. (Fig. 2).

The course of epilepsy seems to be "biphasic", with an active phase, defined by the presence of clinical seizures, of shorter duration around 2 years in 8/14 patients (max 10 years), and a remission phase, defined by the disappearance of seizures, but persisting interictal EEG anomalies. Few patients had uncontrolled epilepsy at the time of inclusion (n = 4) the number of ASMs decreased following a decrease in seizure frequency through age (Fig. 2).

The cerebral MRI was considered normal, except for 4 patients (P2,

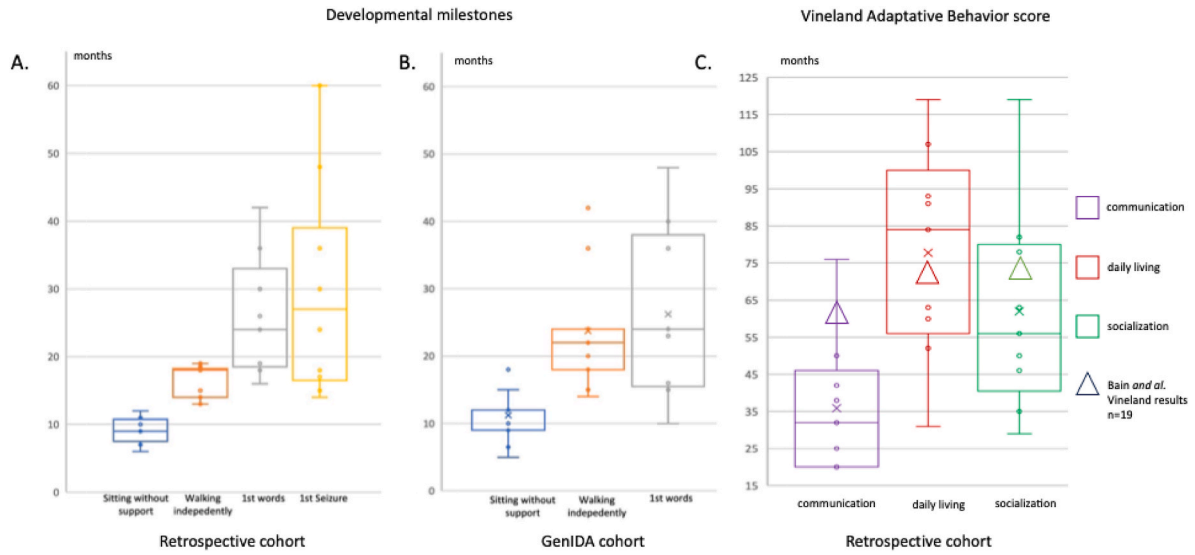

Fig. 1. Neurodevelopment For each patient, interquartile range, mean, median and dispersion for main developmental milestones (sitting, walking and 1st word), age of first seizure is described the same way in our retrospective study from 14 patients (A.) and 14 from the GenIDA study (B.) Main subcategories from our patients' Vineland Adaptive Behavior scores compared to those from 19 patients already reported in Bain *and al.* (C.) X stands for the mean of our cohort and the triangle for the mean of Bain et al. cohort.

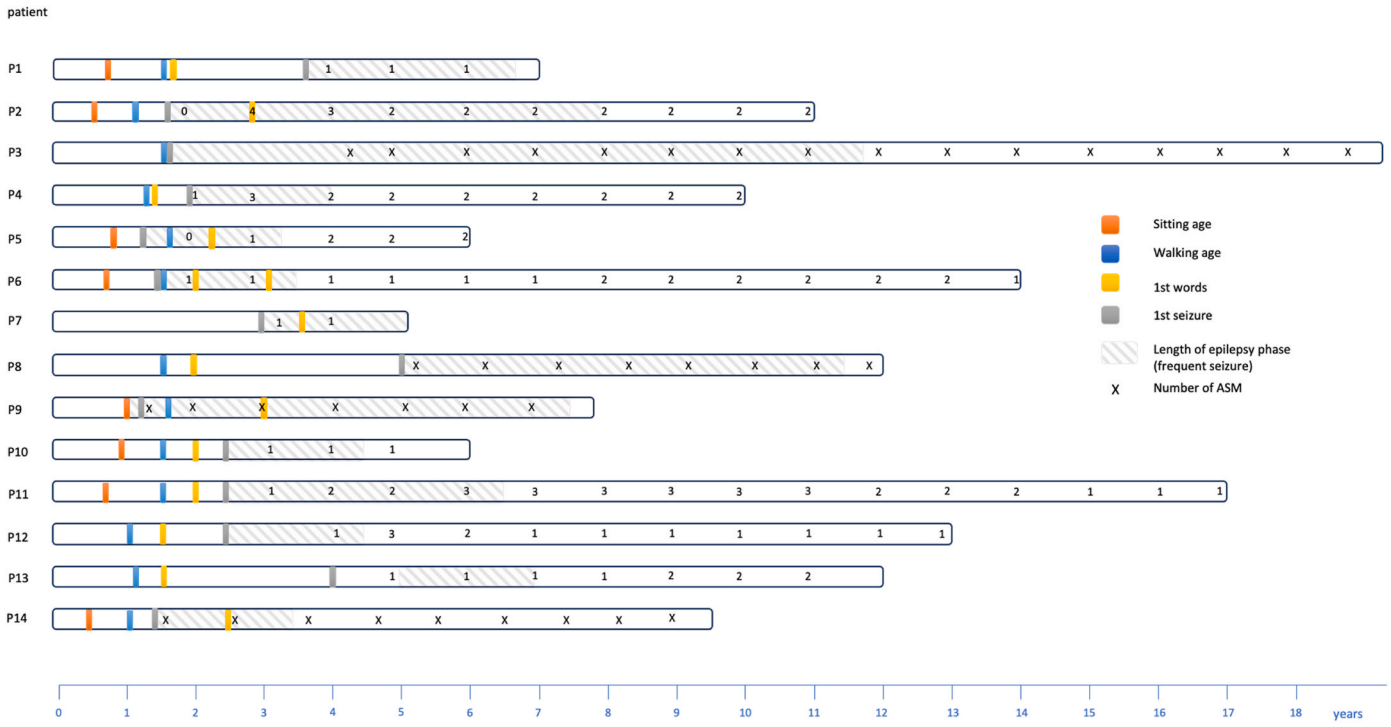

Fig. 2. Neurodevelopmental and epilepsy trajectory described for each of the 14 patients from our retrospective study. In this figure, we have combined developmental milestones (such as sitting age, walking age, and age of first word) with epilepsy characteristics (age of first seizure, length of active seizure phase, and number of antiseizure medications taken per year). The line length for each patient corresponds to their total follow-up period. Patients 3, 8, 12 and 14 were treated with antiseizure medicine with but the duration of each treatment was unknown.

P3, P6, P14) with various non-specific brain anomalies (global atrophy, left temporal atrophy, white matter anomalies, *megacisterna magma*).

C Adaptive functioning: from low average to moderate disability with a heterogeneous profile and language disorder

4 patients (P6, P10, P13, P14) have been previously evaluated with WISC, WPPS, or PEP3 scales, showing mild IDD, or heterogeneous profiles with average or low average scores for visual processing, but below average scores for other processing such as verbal.

9 patients from the retrospective study (P1, P2, P4, P5, P6, P7, P8, P9, and P10) completed from a Vineland Adaptive Behavior Scale (VABS). VABS score ranged from 20 to 76 for communication, daily living from 31 to 119 and socialization from 29 to 119. The global score ranged from low average scores to moderate adaptive levels. The profiles were heterogeneous, with low scores in language abilities,

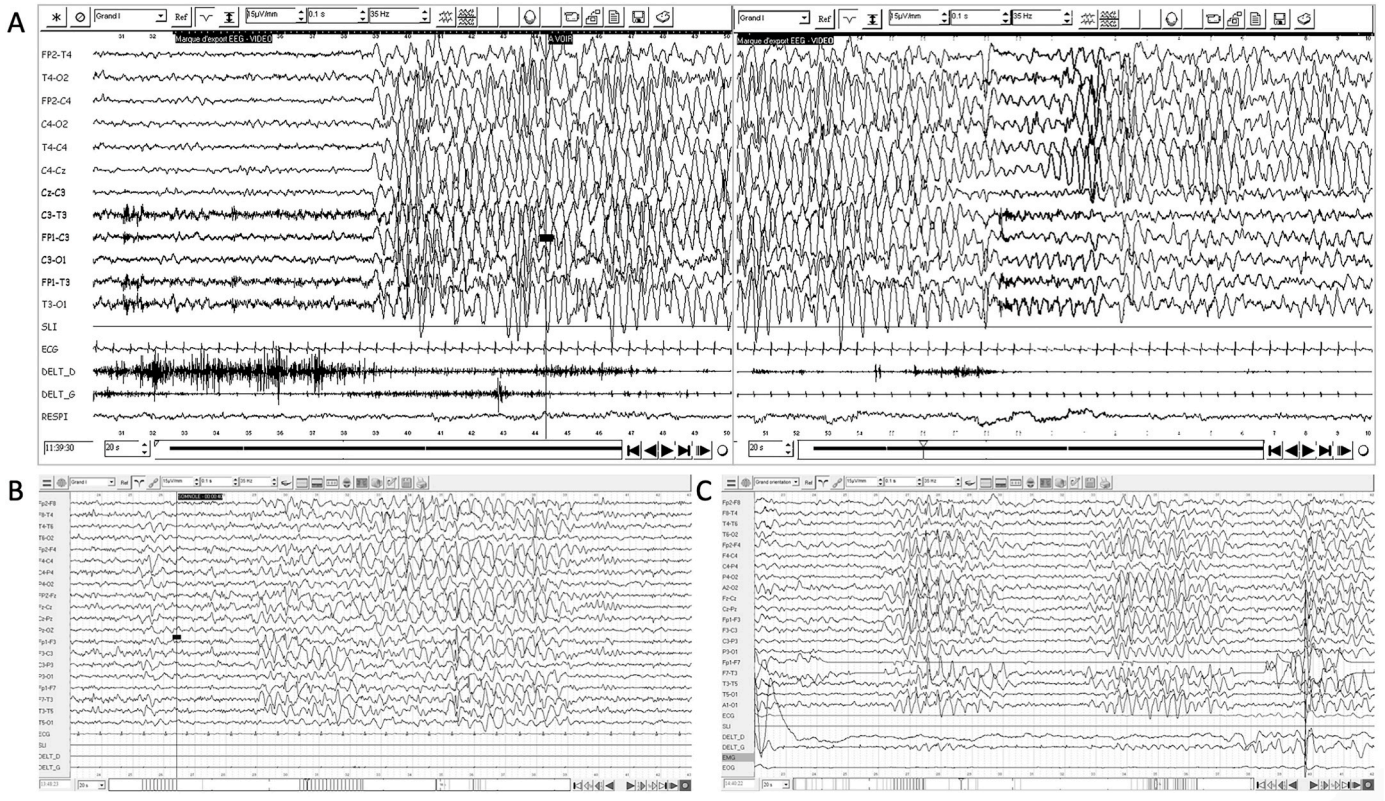

Fig. 3. Epilepsy Typical EEG from a patient with *SLC6A1*-NDD (P8) with intermittent rhythmic delta activity, awake at 3 years (A) 15 years (B) and 19 years (C).

compared to social ability and best scores were obtained in daily living activity. (Fig. 1C).

All the patients had mild to severe expressive and receptive language disorders. Communication regression is often reported by the parents when epilepsy appears.

At both extremes of the spectrum, Patient n°4 (P4) had the global highest scores with high abilities in socialization (119) and daily life (107); this patient presented with epilepsy at the age of 2 years and had a good response to Lamotrigine and Ethosuximide. Patient n°7 (P7) had the lowest score, especially in socialization with a score of 29 but it is important to notice that this patient was born at 30 WG. She also presented with a motor regression and major behavioral disorders, at the age of 3.5 years, concomitant to the first seizures.

D Autism diagnosis screening: frequent repetitive and obsessive behaviors and particular sensory profile, more than typical autism spectrum disorder

Two patients have previously been diagnosed with ASD, by specialized psychiatric teams without precision regarding the specific tests used (P5 and P12).

Seven other patients with clinical suspicion underwent Autism

		Mean	Range	Patients with results above threshold scores
Autism scores	**ADI-R (7)**			
	Communication	3	[0-6]	0
	Social interaction	8	[3-12]	2
	Restricted / repetitive behavior	5,4	[2-11]	6
	SCQ total score (7)	9	[3-17]	1
Behaviour scores	**ABC (7)**			
	Irritability	0,62	[0,2-1,87]	No threshold score
	Lethargy	0,54	[0,38-0,81]	No threshold score
	Stereotypy	0,53	[0,14-1]	No threshold score
	Hyperactivity	1,02	[0,69-1,38]	No threshold score
	Inappropriate speech	0,25	[0-0,75]	No threshold score
	Conners (ADHD index) (8)	19,25	[11-23]	5

Short sensory profile (6)	Number of patients whose results indicate a probable difference	Number of patients whose results indicate a definite difference
Tactile sensitivity	1	0
Taste/smell sensitivity	2	0
Movement sensitivity	0	2
Under responsive/ seeks sensation	0	2
Auditory filtering	3	1
Low energy / weak	1	1
Visual / auditory sensitivity	1	1
Total score	4	1

Fig. 4. Neurodevelopment and behavior Autism scale scores (ADI-R, SCQ), Sensory profile and Behaviour scale scores (ABC, CPRS), and the number of patients that performed this test in brackets for each of them from the retrospective study.

Diagnostic Interview-Revised (ADI-R). This scale covers 3 domains: social interaction issues, communication and language skills, and repetitive and obsessive behavior. Two patients (P1 and P7) had abnormal results for social interaction issues, no patients had significant scores for communication and language skills, and 6 patients had significant scores for repetitive and obsessive behaviors. Only one patient (P1) presented with significant results in two domains (Fig. 4). None of the children in the cohort had sufficient rates in all the subcategories for the diagnosis of ASD with the ADI-R.

Seven patients took the SCQ, and only one of them had a score indicative of ASD(>15) of 17 (P7). This patient (P7) also presented with a significative score for social interaction issues on the ADI-R scale but no definite results for ASD. SCQ and many of the qualitative comments made during the interviews pointed to the relational aspects developed by these patients, including a high degree of stereotypies and increased difficulty in integrating into groups of other children.

Using the short sensory profile, 1 patient presented with a significant peculiar sensory profile (P1) and 4 with probable peculiar sensory anomalies, most concerns were about movement sensitivity, under-responsive/seeking sensation, and auditory filtering (P2, P5, P6, and P8).

These assessments highlighted some autistic features, including restricted repetitive behavior and particular sensory profiles in some of the patients but could not validate a diagnosis of ASD.

E Behavioral assessment

Eight children from the retrospective study completed the CPRS (Fig. 4). Hence, 5 patients presented with scores higher than 15 (P2, P5, P8, P9, P10) that could fit with a diagnosis of ADHD. ABC scores confirmed these observations with mainly impulsivity, distractibility, hyperactivity, and agitation with a higher score in the subscale of hyperactivity and inobservance and a lower score in inappropriate speech in the whole 7 patients tested. During the active phase of epilepsy, parents reported an increase in behavioral problems. These issues may be partially attributed to the use of antiseizure medication, as well as other factors such as environmental influences and improvements in sleep patterns.

F Other Medical Concerns

Some families also reported major sleep disturbances, already been reported in many patients' cohorts from the literature [9,11,27]. Unfortunately, we did not use a specific evaluation form. No clinical commentaries were associated with cardiovascular, respiratory, digestive, or skin impairment.

G. Genetic results

All the variants were shown to be *de novo* and identified using multiple gene panels, exome sequencing, or genome sequencing. Five variants have not been reported in the literature, all being missense variations and considered pathogenic, according to the ACMG classification [17]. This is based on their absence from global population databases, their predicted pathogenicity using different software, and their *de novo* inheritance. In the retrospective study, we reported 3 nonsense variants, 10 missense variants, and 1 amino acid deletion. Seven were in the transmembrane domain (TM) in the protein representation. No recurrent variants were considered in our cohort.

3.2. Cohort 2: 14 international patients from the caregiver reported database GenIDA

Data from 14 other patients have been fulfilled in the GenIDA form, 3 patients have already been included in the first part of the study, hence their GenIDA profiles haven't been studied.

A. Neurodevelopment

Regarding developmental milestones, the median age to sit without support was 12 months (n = 10), walking was achieved between 10 and 48 months (n = 9) (median age 22 months), and first words appeared between 10 and 60 months with a median age of 24 months (n = 11). For 13 patients, caregivers answered about language abilities: 4 of them were not speaking, 3 used words for communication, 3 used complete but often incorrect sentences, and 3 used correct sentences.

According to the completion of the questionnaire, 10 have been diagnosed with IDD (1 profound, 4 severe, 5 moderate) and 2 patients without IDD certified by their parents.

B. Behavior

Most of the patients presented with behavior disorders mostly frustration intolerance, hyperactivity, attention disorder, aggressivity (auto and hetero aggressivity have been reported) and anxiety. About ASD 5 patients were described with ASD diagnosis; ADOS-2 was performed for 2 of them but the information is not complete for the 3 others.

C. Epilepsy

Epilepsy was identified as the primary medical issue. Four patients aged 2 years and 5 months, 4 years and 4 months, 6 years and 6 months, and 10 years and 2 months, did not develop epilepsy at the time of the report. 10/14 patients were described with epilepsy, caregivers gave details about epilepsy with 8 of them with atonic seizures, 6 with absence seizures, 4 having myoclonic seizures, and 2 patients were described with nocturn seizures. None of them were reported with Lennox-Gastaut syndrome or febrile seizures. The treatments mostly reported were Valproic acid (n = 6), Clobazam (n = 3), Ethosuximide (n = 2), Lamotrigine (n = 2), Levetiracetam (n = 2), Cannabidiol, Topiramate, Zonisamide, Briveracetam, Vigabatrin, ketogenic diet was reported only in one patient. Some families reported behavioral adverse effects in the case of corticosteroids, Clobazam, or Valproate treatment. Vigabatrin was associated with ataxia in one patient.

D. Other medical concerns

Tremor was reported in 6 patients that could be accentuated in case of standing up or when lifting the foot off the ground. Fine motor skills problem was reported in 6 patients, 6 patients were also described with abnormal gait. None of them had spastic paraplegia.

Ten caregivers gave information about an MRI with 1 of them having specific anomalies such as mild oedema of spinal roots. Families were also mainly concerned about behavioral difficulties, language delay, and emotional difficulties. and sleep disorders.

4. Discussion

This study is the first one to describe *SLC6A1*-NDD patients combining epilepsy description, multicomponent neurodevelopmental evaluations, and parental clinical questionnaire combined with collected data from worldwide caregivers-reported GenIDA database.

Large cohorts have been described by Dashi et al. with 52 patients and Stefanski et al. with 172 patients but with little detailed clinical information. In 2022, Bain et al. conducted a clinical study comparing 116 patients with data from healthcare providers to 43 patients with data from caregivers using the Simons searchlight register. However, their study was not as comprehensive as ours, particularly in terms of clinical data on epilepsy and reported clinical scores (only SCQ, Vineland, and Social Responsive Scale were included). The SCQ scores in this cohort ranged from 2 to 28, with a mean score of 14.2 (±7.5). Half of the patients scored above 15, and a quarter scored above 22. These scores appear to be higher than those reported in our retrospective cohort of 9

patients, with a mean score of 9 (±5.2), with only one patient exhibiting a score above 15. This suggests that our cohort likely experiences less ASD than the cohort reported by Bain et al. In both cohorts, Vineland Adaptive Behavior Scores have been evaluated. The results were similar regarding socialization but differed significantly in communication, with our cohort showing a mean score of 35.9 compared to 62 in the Bain et al. cohort. Furthermore, our cohort performed better in daily living skills, with a score of 77.8, compared to 68 in the Bain et al. cohort. These differences might reflect the variability associated with patients with *SLC6A1*-related NDD, challenging issues in behavioral assessment when sample sizes are small and/or the limitations of the behavioral assessment.

A specific epileptic phenotype of patients with *SLC6A1*-NDD patients among the genetic generalized syndromes of the young child?

Seizures are a hallmark symptom of *SLC6A1*-NDD with around 90 % of patients experiencing epilepsy [11,29,30]. The most prevalent seizure included absence seizures (typical or atypical), followed by atonic and myoclonic seizures, and seizures typically develop between 8 months and 6 years old [31]. Epilepsy seems to be sensitive to antiseizure medication with 66 % of patients becoming seizure-free while on an ASM in the literature [29]. It may be counterbalanced by Johannessen's recent study showing active epilepsy in 5/11 adult patients with *SLC6A1*-NDD [32].

In our 2 reported cohorts (retrospective study and GenIDA study), 24 patients out of 28 presented with epilepsy and a relative sensibility to ASM since most of them either decreased the number of medications or presented without further seizures in their neurodevelopmental trajectory.

With the increasing number of reported patients, the epilepsy description has been more detailed. The phenotypic spectrum of our patients with *SLC6A1*-NDD fits genetic generalized syndromes of the young child and is most often different from typical myoclonic-atonic epilepsy (MAE) [33]: the patients present with developmental delay especially in language at the time of the diagnosis, the type of seizures is slightly different, with frequent atonic falls, absences, and rare tonic-clonic seizures and no status epilepticus, and the EEG pattern with intermittent rhythmic delta activity focal or generalized, is different than generalized 2–6Hz spike-waves or polyspike waves anomalies in MAE.

The EEG anomalies could persist throughout the life of the patient leading to overtreatment and may be focal sometimes leading to a misdiagnosis of focal epilepsy (Fig. 3). These EEG abnormalities resemble those observed in disorders related to variants in GABA receptor subunits, as well as those with Angelman syndrome due to a 15q11 deletion [34]. These anomalies have also been modelled in GAT-1 knockout mice with spontaneous spike-wave discharges and the absence of seizures was observed as the result of an increased tonic inhibition due to an increase of GABA in the extracellular cleft due to defective GAT-1 function [35,36].

In this cohort, the medication response seems also specific with a good response to Ethosuximide, Valproic Acid, or Lamotrigine, similarly, sensitivity to valproic acid and lamotrigine has also been reported in a cohort of adults with SLC6A1-NDD [32]. These treatments are known to be effective in case of generalized epilepsy. In the caregiver-reported study, these treatments have also been reported but with a lower frequency.

Some patients have experienced a worsening of their condition with Topiramate treatment, both in terms of epilepsy and language development. Adverse effects have also been reported using Vigabatrin or Clobazam in terms of seizure or behavior aggravation.

Neurodevelopmental profile: a spectrum of heterogeneous adaptive functioning, with language disorder.

The adaptive functioning of the 14 reported patients corresponded to low average to moderate IDD, with a heterogeneous profile, as previously reported in other cohorts [3,29,37,38].

Most of the patients presented with global motor development acceptable for the age but delay in language development confirmed after the VABS with communication difficulties. According to the results of the VABS, socialization scores were within the normal range while low scores were reported for the communication domain (Fig. 1C), similar to the communication domain in the ADI-R evaluation (Fig. 4). These difficulties in expression and communication could also be described in the participatory database study from GenIDA.

Most patients had an expressive and receptive language disorder with variable severity. Non-verbal communication techniques such as PECS (Picture Exchange Communication System), Makaton (communication tool with speech, signs, and symbols), or the use of specific numeric tools have been reported effective by some parents. These observations suggest the need for a multidisciplinary approach, in rehabilitation with physical and speech therapists.

Neurodevelopmental and behavioral profiles: some autistic features in half of the patients, and frequent ADHD.

Autistic traits have been broadly described in patients with *SLC6A1*-NDD patients but only a few of these patients underwent a specific assessment [9]. More precisely stereotypies and impaired social interactions are described as common in patients with *SLC6A1*-NDD patients [9,11,30,39,40]. In our retrospective study, 2 patients had a previous diagnosis of ASD, and 7/14 patients were evaluated using ADI-R, based on clinical suspicion; in the GenIDA study 5 patients were described with ASD but only 2 of them underwent ADI-R evaluation. Half of the patients from the retrospective study presented with peculiar features of this wide spectrum, with frequent stereotypies, repetitive obsessive disorder, and peculiar sensory profile with movement sensitivity, under-responsiveness, and auditory filtering.

Secondary to the families' interviews regarding behavioral disorders, most of the patients (5/7) presented with ADHD phenotype using the CPRS. Very few patients have a specific medical treatment, but the interviews highlighted the importance of regular psychiatric follow-up. Anxiety was also frequently reported by the parents but not specifically investigated. Some families also reported major sleep disturbances already reported in many patients' cohorts from the literature [9,11,29], unfortunately, we did not use a specific evaluation form.

With the wide age cohort reported hereby, we could also assess the global trajectory of patients with *SLC6A1*-NDD. The oldest patient of our retrospective study now aged 45 years, could work in an employment assistance center. In a recent report from Johannessen et al., adult-reported patients presented mainly with moderate to severe IDD, probably more severe in adult patients. The higher rate of severe NDD in adults could be a result of selection bias. Probably genetic testing is more likely to be carried out in cases of severe disease [41].

One limitation of our evaluation could be the teleconference evaluation of the patients due to the pandemic situation and the fulfilment of the questionnaire by the parents [28].

SLC6A1-NDD, a developmental encephalopathy with epilepsy: factors contributing to the variable severity?

The genotype-phenotype correlation is still debated in *SLC6A1*-NDD [9,12]. Recent publications have reported the variability of the phenotype especially in families with inherited variants [13], hypothesizing the existence of other genetic and environmental factors. Some recent publications tried to correlate the severity of epilepsy and IDD with GABA uptake without significant results.

Our small cohort represents a part of the spectrum of *SLC6A1*-NDD, with an early epileptic presentation. The impact of epilepsy, and interictal EEG anomalies, in the developmental trajectory of patients with *SLC6A1*-NDD remains unclear, and this field must be investigated more precisely, with accurate studies of individual developmental trajectories, and following EEG, as it may be a biomarker.

It is still challenging to classify SLC6A1-NDD as a specific developmental and epileptic encephalopathy. We preferred to use the term "developmental encephalopathy with epilepsy" as we could not demonstrate a developmental stagnation or regression, during the active phase of the epilepsy. Further developmental assessments at various

time points during the developmental trajectory and the different phases of epilepsy are necessary to understand the interaction of these processes more accurately. Moreover, the question of the impact of treatments during these important periods for cognition also remains to be clarified [42].

4.1. Comparison to the GenIDA participatory database study and limitations

In the retrospective French study of 14 SLC6A1-NDD patients, we encountered some limitations. Caregivers were interviewed by a trained pediatric neurologist, and medical files were also studied. However, these interviews took place during the pandemic, so they were conducted via teleconsultation, possibly resulting in less interaction. During this process, a professional was present to address any questions and misunderstandings from the caregivers.

In the GenIDA section, establishing a correlation was challenging. The questionnaire consisted mainly of closed questions applicable to all genetic NDD cases, which could lead to inaccuracies. For example, one child, who at 2.5 years old didn't walk or speak much and had low social interaction, was described by the parents as not having an IDD. This could be due to the child's young age, the inability to provide specific tests at that age, or the parents misunderstanding their child's abilities.

The two questionnaires also helped us identify certain specific issues from the parent's perspective, with many of them highlighting trembling or sleep disorders. Although these specific impairments were not precisely studied in our evaluation, they should be addressed in the next report.

Both cohorts were extensively described on certain specific points (such as epilepsy or behavior evaluation), but due to their small size, it's challenging to establish correlations on a broader scale. Large-scale standardized studies are necessary to enhance our understanding of the natural history of SLC6A1-NDD patients.

5. Conclusion

We highlighted specific characteristics of SLC6A1-NDD patients, using a description of epilepsy and multicomponent neurodevelopmental scales, interviews with families, compared to data reported by caregivers in an international database.

We observed a specific epileptic phenotype within the genetically generalized syndromes of young children, with fewer seizures over time in most cases. Their neurodevelopmental profile combines a spectrum of heterogeneous adaptive functioning, with language disorder, some autistic features in half of them, and frequent ADHD and anxiety.

Parental reports and qualitative and quantitative assessments have helped us to pinpoint the main domains of concerns, that must be explored in a young patient, such as behavior and sleep quality. They also alert on the special educational and rehabilitation needs according to the profile of their child, besides the symptomatic treatments.

Apart from symptomatic treatments, some therapeutics could emerge in this NDD secondary to the pathophysiological implication of *SLC6A1* such as 4-phenylbutyrate [43]. Further studies on the natural history and precise characterization of patient trajectories will enable us to identify prognostic factors and relevant therapeutic targets.

Declaration of competing interest

None of the authors have any conflicts of interest related to this study to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2025.01.003.

References

[1] K.K. Madsen, G.H. Hansen, E.M. Danielsen, A. Schousboe, The subcellular localization of GABA transporters and its implication for seizure management, Neurochem. Res. 40 (2) (2015) 410–419, https://doi.org/10.1007/s11064-014-1494-9.

[2] J. Wang, Y. Wang, L. Wang, W.Y. Chen, M. Sheng, The diagnostic yield of intellectual disability: combined whole genome low-coverage sequencing and medical exome sequencing, BMC Med. Genom. 13 (1) (2020) 70, https://doi.org/10.1186/s12920-020-0726-x.

[3] G.L. Carvill, J.M. McMahon, A. Schneider, et al., Mutations in the GABA transporter SLC6A1 cause epilepsy with myoclonic-atonic seizures, Am. J. Hum. Genet. 96 (5) (2015) 808–815, https://doi.org/10.1016/j.ajhg.2015.02.016.

[4] K.M. Johannesen, E. Pérez-Palma, G. Rubboli, Editorial: SLC6A1: the past, present and future, Front. Neurosci. 17 (2023) 1289821, https://doi.org/10.3389/fnins.2023.1289821.

[5] Autism Spectrum Disorder Working Group of the Psychiatric Genomics Consortium, BUPGEN, Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium, et al., Identification of common genetic risk variants for autism spectrum disorder, Nat. Genet. 51 (3) (2019) 431–444, https://doi.org/10.1038/s41588-019-0344-8.

[6] F fen Yuan, X. Gu, X. Huang, Y. Zhong, J. Wu, SLC6A1 gene involvement in susceptibility to attention-deficit/hyperactivity disorder: a case-control study and gene-environment interaction, Prog. Neuro Psychopharmacol. Biol. Psychiatr. 77 (2017) 202–208, https://doi.org/10.1016/j.pnpbp.2017.04.015.

[7] GROUP Investigators, E. Rees, J. Han, et al., De novo mutations identified by exome sequencing implicate rare missense variants in SLC6A1 in schizophrenia, Nat. Neurosci. 23 (2) (2020) 179–184, https://doi.org/10.1038/s41593-019-0565-2.

[8] J.A. López-Rivera, E. Pérez-Palma, J. Symonds, et al., A catalogue of new incidence estimates of monogenic neurodevelopmental disorders caused by de novo variants, Brain 143 (4) (2020) 1099–1105, https://doi.org/10.1093/brain/awaa051.

[9] K. Goodspeed, L.R. Mosca, N.C. Weitzel, et al., A draft conceptual model of SLC6A1 neurodevelopmental disorder, Front. Neurosci. 16 (2023) 1026065, https://doi.org/10.3389/fnins.2022.1026065.

[10] H. Dahshi, S. Kalvakuntla, M. Lee, K. Goodspeed, Beyond the diagnosis: evaluation of quality-of-life measures and family functioning in slc6a1-related neurodevelopmental disorder, Pediatr. Neurol. 155 (2024) 160–166, https://doi.org/10.1016/j.pediatrneurol.2024.03.030.

[11] J.M. Bain, L.G. Snyder, K.L. Helbig, D.D. Cooper, W.K. Chung, K. Goodspeed, Consistency of parent-report SLC6A1 data in Simons searchlight with provider-based publications, J. Neurodev. Disord. 14 (1) (2022) 40, https://doi.org/10.1186/s11689-022-09449-7.

[12] A. Stefanski, E. Pérez-Palma, T. Brünger, et al., SLC6A1 variant pathogenicity, molecular function, and phenotype: a genetic and clinical analysis, Brain (August 30, 2023) awad292, https://doi.org/10.1093/brain/awad292. Published online.

[13] B. Kassabian, C.D. Fenger, M. Willems, et al., Intrafamilial variability in SLC6A1-related neurodevelopmental disorders, Front. Neurosci. 17 (2023) 1219262, https://doi.org/10.3389/fnins.2023.1219262.

[14] D.B. Silva, M. Trinidad, A. Ljungdahl, et al., Haploinsufficiency underlies the neurodevelopmental consequences of SLC6A1 variants, Am. J. Hum. Genet. (May 2024) S0002929724001629, https://doi.org/10.1016/j.ajhg.2024.04.021. Published online.

[15] S. Kalvakuntla, M. Lee, W.K. Chung, et al., Patterns of developmental regression and associated clinical characteristics in SLC6A1-related disorder, Front. Neurosci. 17 (2023) 1024388, https://doi.org/10.3389/fnins.2023.1024388.

[16] S. Beniczky, W.O. Tatum, H. Blumenfeld, et al., Seizure semiology: ILAE glossary of terms and their significance, Epileptic Disord. 24 (3) (2022) 447–495, https://doi.org/10.1684/epd.2022.1430.

[17] S. Richards, N. Aziz, S. Bale, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (5) (2015) 405–424, https://doi.org/10.1038/gim.2015.30.

[18] N. Beail, Utility of the Vineland adaptive behavior scales in diagnosis and research with adults who have mental retardation, Ment. Retard. 41 (4) (2003) 286–289, https://doi.org/10.1352/0047-6765(2003)41<286:UOTVAB>2.0.CO;2.

[19] A. De Bildt, D. Kraijer, S. Sytema, R. Minderaa, The psychometric properties of the Vineland adaptive behavior scales in children and adolescents with mental retardation, J. Autism Dev. Disord. 35 (1) (2005) 53–62, https://doi.org/10.1007/s10803-004-1033-7.

[20] C. Lord, M. Rutter, A. Le Couteur, Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders, J. Autism Dev. Disord. 24 (5) (1994) 659–685, https://doi.org/10.1007/BF02172145.

[21] W. Dunn, C. Brown, Factor analysis on the Sensory Profile from a national sample of children without disabilities, Am. J. Occup. Ther. 51 (7) (1997) 490–495, https://doi.org/10.5014/ajot.51.7.490. ; discussion 496-499.

[22] S.R. Chesnut, T. Wei, L. Barnard-Brak, D.M. Richman, A meta-analysis of the social communication questionnaire: screening for autism spectrum disorder, Autism 21 (8) (2017) 920–928, https://doi.org/10.1177/1362361316660065.

[23] P. Fumeaux, C. Mercier, S. Roche, et al., Validation of the French version of Conners' parent rating scale revised, short version: factorial structure and reliability, Can. J. Psychiatr. 61 (4) (2016) 236–242, https://doi.org/10.1177/0706743716635549.

[24] M. Aman, N. Singh, A. Stewart, C. Field, The Aberrant Behavior Checklist: a behavior rating scale for the assessment of treatment effects, Am. J. Ment. Defic. 89 (1985) 485–491.
[25] B. Durand, E. Schaefer, P. Burger, et al., Neurocognitive and neurobehavioral characterization of two frequent forms of neurodevelopmental disorders: the DYRK1A and the Wiedemann–Steiner syndromes, Clin. Genet. 102 (4) (2022) 296–304, https://doi.org/10.1111/cge.14190.
[26] B. Engel-Yeger, R. Hardal-Nasser, E. Gal, Sensory processing dysfunctions as expressed among children with different severities of intellectual developmental disabilities, Res. Dev. Disabil. 32 (5) (2011) 1770–1775, https://doi.org/10.1016/j.ridd.2011.03.005.
[27] P. Salehi, L. Herzig, G. Capone, A. Lu, A.P. Oron, S. Kim, Comparison of aberrant behavior checklist profiles across prader–willi syndrome, down syndrome, and autism spectrum disorder, Am. J. Med. Genetics Pt A 176 (12) (2018) 2751–2759, https://doi.org/10.1002/ajmg.a.40665.
[28] P. Burger, F. Colin, A. Strehle, et al., GenIDA: an international participatory database to gain knowledge on health issues related to genetic forms of neurodevelopmental disorders, J. Neural. Transm. 130 (3) (2023) 459–471, https://doi.org/10.1007/s00702-022-02569-3.
[29] K.M. Johannesen, E. Gardella, T. Linnankivi, et al., Defining the phenotypic spectrum of *SLC6A1* mutations, Epilepsia 59 (2) (2018) 389–402, https://doi.org/10.1111/epi.13986.
[30] A. Kahen, H. Kavus, A. Geltzeiler, et al., Neurodevelopmental phenotypes associated with pathogenic variants in *SLC6A1*, J. Med. Genet. 59 (6) (2022) 536–543, https://doi.org/10.1136/jmedgenet-2021-107694.
[31] K. Goodspeed, E. Pérez-Palma, S. Iqbal, et al., Current knowledge of SLC6A1-related neurodevelopmental disorders, Brain Commun. 2 (2) (2020) fcaa170, https://doi.org/10.1093/braincomms/fcaa170.
[32] K.M. Johannesen, J. Nielsen, A. Sabers, et al., The phenotypic presentation of adult individuals with SLC6A1-related neurodevelopmental disorders, Front. Neurosci. 17 (2023) 1216653, https://doi.org/10.3389/fnins.2023.1216653.
[33] N. Specchio, E.C. Wirrell, I.E. Scheffer, et al., International league against epilepsy classification and definition of epilepsy syndromes with onset in childhood: position paper by the ILAE task force on nosology and definitions, Epilepsia 63 (6) (2022) 1398–1442, https://doi.org/10.1111/epi.17241.
[34] P. Maillard, S. Baer, É. Schaefer, et al., Molecular and clinical descriptions of patients with GABA $_A$ receptor gene variants (*GABRA1* , *GABRB2* , *GABRB3* , *GABRG*): A cohort study, review of literature, and genotype–phenotype correlation, Epilepsia 63 (10) (2022) 2519–2533, https://doi.org/10.1111/epi.17336.
[35] D.A. Richards, T. Lemos, P.S. Whitton, N.G. Bowery, Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study, J. Neurochem. 65 (4) (2002) 1674–1680, https://doi.org/10.1046/j.1471-4159.1995.65041674.x.
[36] D. Belelli, N.L. Harrison, J. Maguire, R.L. Macdonald, M.C. Walker, D.W. Cope, Extrasynaptic GABA $_A$ receptors: form, pharmacology, and function, J. Neurosci. 29 (41) (2009) 12757–12763, https://doi.org/10.1523/JNEUROSCI.3340-09.2009.
[37] A. Rauch, D. Wieczorek, E. Graf, et al., Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study, Lancet 380 (9854) (2012) 1674–1682, https://doi.org/10.1016/S0140-6736(12)61480-9.
[38] S. Devries, M. Mulder, J.G. Charron, J.W. Prokop, P.R. Mark, SLC6A1 G443D associated with developmental delay and epilepsy, Cold Spring Harb. Mol. Case Stud. 6 (4) (2020) a005371, https://doi.org/10.1101/mcs.a005371.
[39] F.P. Fischer, A.S. Kasture, T. Hummel, S. Sucic, Molecular and clinical repercussions of GABA transporter 1 variants gone amiss: links to epilepsy and developmental spectrum disorders, Front. Mol. Biosci. 9 (2022) 834498, https://doi.org/10.3389/fmolb.2022.834498.
[40] P.K. Ahring, V.W.Y. Liao, E. Gardella, et al., Gain-of-function variants in *GABRD* reveal a novel pathway for neurodevelopmental disorders and epilepsy, Brain 145 (4) (2022) 1299–1309, https://doi.org/10.1093/brain/awab391.
[41] I. Krey, K. Platzer, J.R. Lemke, Monogenetic epilepsies and how to approach them in 2022, Med. Genet. 34 (3) (2022) 201–205, https://doi.org/10.1515/medgen-2022-2143.
[42] F.M.C. Besag, M.J. Vasey, Neurocognitive effects of antiseizure medications in children and adolescents with epilepsy, Paediatr Drugs 23 (3) (2021) 253–286, https://doi.org/10.1007/s40272-021-00448-0.
[43] G. Nwosu, F. Mermer, C. Flamm, et al., 4-Phenylbutyrate restored γ-aminobutyric acid uptake and reduced seizures in *SLC6A1* patient variant-bearing cell and mouse models, Brain Commun. 4 (3) (2022) fcac144, https://doi.org/10.1093/braincomms/fcac144.