Journal of Neurology (2019) 266:1439–1448
https://doi.org/10.1007/s00415-019-09280-6

ORIGINAL COMMUNICATION

Screening of *SLC2A1* in a large cohort of patients suspected for Glut1 deficiency syndrome: identification of novel variants and associated phenotypes

Barbara Castellotti[1] · Francesca Ragona[2] · Elena Freri[2] · Roberta Solazzi[2] · Stefano Ciardullo[1] · Giovanni Tricomi[3] · Anna Venerando[1] · Barbara Salis[2] · Laura Canafoglia[4] · Flavio Villani[5] · Silvana Franceschetti[4] · Nardo Nardocci[2] · Cinzia Gellera[1] · Jacopo C. DiFrancesco[4,6] · Tiziana Granata[2]

Received: 20 September 2018 / Revised: 4 March 2019 / Accepted: 13 March 2019 / Published online: 20 March 2019

Abstract

Glucose transporter type 1 deficiency syndrome (Glut1 DS) is a rare neurological disorder caused by impaired glucose delivery to the brain. The clinical spectrum of Glut1 DS mainly includes epilepsy, paroxysmal dyskinesia (PD), developmental delay and microcephaly. Glut1 DS diagnosis is based on the identification of hypoglycorrhachia and pathogenic mutations of the *SLC2A1* gene. Here, we report the molecular screening of *SLC2A1* in 354 patients clinically suspected for Glut1 DS. From this cohort, we selected 245 patients for whom comprehensive clinical and laboratory data were available. Among them, we identified 19 patients carrying nucleotide variants of pathological significance, 5 of which were novel. The symptoms of onset, which varied from neonatal to adult age, included epilepsy, PD or non-epileptic paroxysmal manifestations. The comparison of the clinical features between the 19 *SLC2A1* mutated and the 226 non-mutated patients revealed that the onset of epilepsy within the first year of life (when associated with developmental delay or other neurological manifestations), the association of epilepsy with PD and acquired microcephaly are more common in mutated subjects. Taken together, these data confirm the variability of expression of the phenotypes associated with mutation of *SLC2A1* and provide useful clinical tools for the early identification of subjects highly suspected for the disease.

Keywords Glut1 deficiency · *SLC2A1* · Hypoglycorrhachia · Epilepsy · Movement disorder · Intellectual disability · Developmental delay

Introduction

Glucose transporter type 1 deficiency syndrome (Glut1 DS, OMIM 606777) is a rare neurological disorder caused by impaired glucose delivery to the brain [1]. It results from haploinsufficiency of the *SLC2A1* gene encoding Glut1, the main protein responsible for glucose transport across the

Barbara Castellotti and Francesca Ragona equally contributed to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00415-019-09280-6) contains supplementary material, which is available to authorized users.

✉ Jacopo C. DiFrancesco
jacopo.difrancesco@gmail.com

✉ Tiziana Granata
tiziana.granata@istituto-besta.it

1 Unit of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

2 Department of Pediatric Neuroscience, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

3 Unit of Infancy and Adolescence Neuropsychiatry, Azienda Unità Sanitaria Locale della Romagna, Cesena, Italy

4 Clinical Neurophysiology and Epilepsy Center, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

5 Clinical and Experimental Epileptology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

6 Department of Neurology, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy

blood–brain barrier [2]. It was first described in patients presenting with infantile seizures, developmental delay, acquired microcephaly, persistent hypoglycorrhachia and a complex movement disorder consisting of ataxia and spasticity [1]. Since then, the clinical spectrum associated with Glut1 DS has been extensively broadened and over the years many other clinical manifestations have been described, such as paroxysmal dyskinesia (PD) with or without epilepsy, alternating hemiplegia, and migraine [3, 4].

Epilepsy is present in approximately 90% of patients [5] and, beyond the classic drug-resistant infantile seizures, the epileptic phenotype includes both focal and generalized epilepsies. Several authors have recently shown that 10% of early-onset absence epilepsies [6, 7], 5% of myoclonic-astatic epilepsies [8] and 1% of idiopathic generalized epilepsies [9] are caused by mutations of the *SLC2A1* gene, although these results were not confirmed by subsequent studies [10, 11].

Diagnosis is confirmed by the evidence of hypoglycorrhachia [12] and loss-of-function mutations in the *SLC2A1* gene. Providing an early diagnosis is crucial for patients, as it allows a prompt initiation of the ketogenic diet, which has been proved effective in the management of seizures and movement disorders in Glut1 DS patients [13, 14].

We performed the genetic analysis of *SLC2A1* gene in 354 patients clinically suspected for Glut1 DS, and identified the presence of nucleotide variants of pathological significance in 19 subjects. The aims of our study were: (1) to summarize the clinical characteristics of 19 patients carrying *SLC2A1* mutations, (2) to describe the clinical features of 5 patients carrying novel mutations and (3) to compare the clinical characteristics of mutated patients with those of non-mutated.

Patients and methods

Ethical issues

Biological samples from patients and their relatives were obtained for analysis, research and storage, after the acquisition of written informed consent according to international guidelines and approved by the Internal Institutional Ethics Committee.

All human studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Patients

We retrospectively reviewed all the patients who underwent the genetic screening of *SLC2A1* in our Institution between January 2005 and December 2016. The analysis was requested according to the suspicions of Glut1 DS, for one or more of the following indications: epilepsy, PD or other non-epileptic paroxysmal events. The molecular screening of *SLC2A1* was carried out in 354 patients. From this cohort, we selected 245 patients for whom clinical and laboratory data were fully available. From our in house database, we collected the following information: gender, age at Glut1 DS diagnosis, characteristics of the epileptic features (including age of onset, seizure type and EEG characteristics), drug resistance, paroxysmal dyskinesia (onset and type), other symptoms (onset and diagnosis at the clinical observation), CSF data (CSF glucose/glycemia ratio) and genetic data (result of the *SLC2A1* gene screening).

Molecular studies

Genomic DNA was extracted from peripheral blood lymphocytes, according to standard procedures. Written informed consent for DNA analysis was obtained from all patients and family members. DNA samples were screened for mutations in the *SLC2A1* gene (exons 1–10). Exons and intron–exon boundaries were analyzed by direct sequence analysis using an automated sequencing system (ABI 3130 XL). The primers are available on request. Patients negative for point mutations in the gene, with hypoglycorrhachia and/or the presence of common polymorphisms in homozygous condition indicating a possible presence of a heterozygous deletion, were also screened for exon deletion or duplication by multiplex ligation-dependent probe amplification (MLPA). MLPA was performed using the SALSA MLPA kit P138-*SLC2A1* (MRC-Holland). We have also performed segregation analysis in parents of patients carrying variants in *SLC2A1* gene. Only in patient # 19 parents' DNA was not available for segregation studies.

Nucleotides and amino acid residues were numbered according to the reference gene sequence of the transcript GenBank (NCBI): *SLC2A1* (Homo sapiens NM_006516, NP_006507). Sequence variations and predicted protein changes were described according to nomenclature recommendations (http://www.hgvs.org/mutnomen/recs.html). Segregation of genetic variants was analyzed through validation in all available family members. Frequencies of novel missense variants were determined by comparison with The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), the NCBI dbSNP132ver (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the Exome Variant Server (http://evs.gs.washington.edu/EVS/). In silico analysis of missense variants was performed using the PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and SIFT prediction test (http://sift.jcvi.org/). ASSP algorithm (http://wangcomputing.com/assp/) was used for alternative splice site prediction as the novel variant occurred close to the 5′ splice site, and ESE Finder Software 3.0 (http://rulai.cshl.edu/

cgi-bin/tools/ESE3/esefinder.cgi?process=home) for evaluating the disruption of putative exonic splicing enhancers (ESEs), which are binding sites for human SR proteins SF2/ASF, SC35, SRp40 and SRp55.

Statistical analysis

Statistical analysis was conducted using STATA/IC version 15. Descriptive statistics were expressed as means and standard deviations for continuous variables and as percentages for categorical variables. Comparisons of continuous data were performed with t test and those of categorical data with chi-squared test or Fisher's exact test. Variables that were found to be statistically significant were analyzed using the logistic regression. A p value ≤ 0.05 was considered statistically significant.

Results

From January 2005 to December 2016, 354 patients were analyzed by *SLC2A1* sequencing. We detected sequence variants in 19 patients, belonging to 18 families (5.4%), and 5 of these variants were novel (Table 1).

Clinical features of the 19 *SLC2A1*-mutated patients

The presenting symptom or the reason for referring was epilepsy in 16 patients (84.2%) and movement disorders in 3. The mean age at molecular analysis was 16.0 ± 7.6 years. The series includes 17 sporadic cases and 1 familial case. Fifteen out of the 17 sporadic cases carried a de novo variant (i.e. not present in parents), in one patient (# 19) parents' DNA was not available for segregation studies and in one patient (#12) the identified variant was also present in the asymptomatic father. One case was familial because the identified variant was present in the proband and in her symptomatic father (#15 and #16). All patients were born to non-consanguineous parents; pregnancy, delivery and the neonatal period were uneventful in all. Clinical, biochemical and genetic features of patients are detailed in Table 1. Patients #4, 5, 11, 17 have been already reported [15, 16]. The clinical features of the five patients carrying *SLC2A1* novel variants are reported in the Supplementary material.

Patients presenting with seizures

The mean age at seizure onset was 2.4 years (range 2 months–9 years). Eight patients (#1–7, 15) had epilepsy onset in the first year of life characterized by myoclonic (6 infants), convulsive (1), or focal seizures (1). At the time of epilepsy onset, all these patients had other symptoms (i.e. developmental delay, or abnormalities of eye movements, or hypotonia).

In 8 patients (#8–14, 16), epilepsy onset was after the first year of age. The absence was the most common seizure type (seven cases), preceded by febrile seizures in two children. One patient had focal seizures. At the onset of epilepsy, the large majority of patients had associated symptoms: intellectual disabilities of various degree or specific learning disability, microcephaly, headache, dystonia, spasticity and ataxia. In six patients, seizure frequency was increased by fasting. During the course of the disease, paroxysmal dyskinesia appeared in 10 out of the 16 patients with epilepsy, after a mean interval of 5.9 ± 2.2 years. PD featured as PED in seven patients, as PNKD in two and as PKD in one. At molecular diagnosis, all the mutated patients had a complex clinical picture characterized by the association of epilepsy with at least one of the following symptoms: MD, microcephaly, headache, ataxia, myoclonus, spasticity, dystonia and intellectual disability of various severity.

Patients presenting with movement disorders

Paroxysmal movement disorder was the first symptom in three patients (PED in two and PNKD in one), with an age at onset ranging between 4 and 13 years (# 14–16). In all cases, symptoms worsened by fasting. Additional features, variably associated in the different patients (see Table 1) were microcephaly, headache intellectual disability, ataxia, spasticity and dystonia. None of these patients experienced epileptic seizures during the disease course (present age ranging from 21 to 42 years).

Clinical features of the 226 *SLC2A1*-non-mutated patients

The mean age at molecular analysis was 12.5 ± 11.8 years. The main reason for referring was epilepsy (168 patients) that was associated with PD in 9. In most patients with epilepsy as a prominent symptom, the onset of seizure was after the first year of life (135 patients). Two out of these patients experienced PD during the follow-up. Epilepsy was characterized by generalized seizures with absences and/or myoclonic seizures, associated with diffuse spikes and waves at in 96 patients and by generalized or focal seizures without spike and waves in 37. Thirty-three patients (14.5%) were referred for epilepsy which had started within the first year of life, either as epileptic encephalopathy, or myoclonic or focal epilepsy. In 73% of patients, epilepsy was associated with developmental delay and with PD in seven.

Twenty-nine patients underwent genetic analysis for the presence of PD, and 17 for other non-epileptic paroxysmal events such as headache, alternating hemiplegia, episodic ataxia. Finally, in 12 patients, the genetic analysis was

Table 1 Clinical, laboratory and genetic data in *SLC2A1* mutated patients

	Pt	Sex	Present age (year)	Age at diagnosis (year)	Epilepsy				Paroxysmal dyskinesia		Other symptoms		Glycorrachia/glycemia (ratio)	CSF lactate	*SLC2A1* mutation
					Onset (year)	Seizure type	EEG	DR	Onset (year)	Type	At onset	At diagnosis			
Epilepsy onset < 1 year	#1	F	12	5.4	0.13	FS GTC	G	No	4.5	PED	None	Pyramidal signs, MC	31/79 (0.39)	7.2	c.457C > T p.Arg153Cys Pascual et al. (2002) Segregation not possible. Adopted child
	#2	M	1.5	0.4	0.25	MYO	SW	Yes	-	-	Mild DD, POM	Moderate DD hypotonia, POM	23/90 (0.25)	7.9	c.85_89delAACAC p.Trp29fs*57 Novel De novo
	#3	F	6	6	0.25	MYO eyelid	SW	Yes	-	-	Mild DD	Mild ID, feeling hunger, fatigue	32/68 (0.47)	8.1	c.692T > C p.Leu231Pro Novel De novo
	#4	F	31	22	0.3	MYO, FC	SW	Yes	10	PED	DD	Pyramidal signs, MC, moderate ID	31/81 (0.38)	7.6	c.376C > T p.Arg126Cys [16] De novo
	#5	F	29	19	0.5	MYO, ABS	SW	No	7	PED	DD, dysarthria	Ataxia, spasticity, dystonia, MC, severe ID	26/77 (0.33)	7.3	c.847C > T p.Gln283Ter [16] De novo
	#6	M	7	3.3	0.42	MA	SW	No	–	–	Mild DD POM	MC, mild ID	NA	NA	c.1177G > A p.Glu393Lys Novel De novo
	#7	M	15	7.3	0.83	FM	F	No	4.8	PNK, D	Mild DD	Dyspraxia	NA	NA	c.1278delG + 1delG Splice site Novel De novo

Table 1 (continued)

	Pt	Sex	Present age (year)	Age at diagnosis (year)	Epilepsy				Paroxysmal dyskinesia		Other symptoms		Glycorrachia/glycemia (ratio)	CSF lactate	*SLC2A1* mutation
					Onset (year)	Seizure type	EEG	DR	Onset (year)	Type	At onset	At diagnosis			
Epilepsy onset > 1 year	#8	F	13	5.7	1.5	ABS	–	Yes	5	PED	Mild DD	Ataxia, DD, MC, borderline cognitive functioning	NA	NA	c.418G > A p.Val140Met [7] De novo
	#9	F	34	26	2	FM, GTC	F	No	13	PED	Not known	Not known	NA	NA	c.493G > A p.Val165Ile Urbizu et al. [22] De novo
	#10	M	11	4.6	2.5	ABS	NA	NA	3	PED	Mild DD	Dystonia, mild ID	NA	NA	c.1198C > T p.Arg400Cys Liu et al. [23] De novo
	#11	F	18	12.2	4	ABS- FS	SW	Yes	12	PKD	None	HA, borderline cognitive abilities, feeling Hunger	NA	NA	c.823G > A p.Ala275Thr [4] De novo
	#12	F	12	10	6.5	ABS	SW	No	-	-	SLD	SLD, feeling hunger, fatigue	44/84 (0.52)	NA	C.26C > T p.Thr9Met Novel Inherited from healthy father
	#13	F	23	18.4	6	ABS	SW	Yes	17	PNKD	ataxia, DD	Ataxia, ID mild ID, MC	38/ 92 (0.41)	7.8	c.274C > T p.Arg92Trp Schneider et al. [24] De novo
	#14	F	11	9	9	ABS, FS	SW	No	–	–	None	Mild ID, HA	43/100 (0.43)	8.8	c.940G > A p.Gly314Ser [4] De novo

Table 1 (continued)

	Pt	Sex	Present age (year)	Age at diagnosis (year)	Epilepsy				Paroxysmal dyskinesia		Other symptoms		Glycorrachia/glycemia (ratio)	CSF lactate	*SLC2A1* mutation
					Onset (year)	Seizure type	EEG	DR	Onset (year)	Type	At onset	At diagnosis			
Familial case	#15	M	42	39.3	0,5	MYO—ABS	SW	No	8	PED	None	None	NA	NA	c.101A > G p.Asn34Ser [13]
	#16	F	10	6.6	4.5	ABS	SW	NA	-	-	Motor clumsiness	d, MC	NA	NA	c.101A > G p.Asn34Ser [13] Inherited from affected father (#15)
PD	#17	F	30	19	–	–	–	–	4	PNKD	MC, d, DD	a, sp, d, MC, severe ID	26/72 (0.36)	7.5	c.144G > A p.Trp48Ter [16] De novo
	#18	M	21	17.4	–	–	–	No	13	PED	Mild DD	a, mild ID, HA	46/87 (0.52)	8.9	c.1372C > T p.Arg458Trp [6] De novo
	#19	M	42	38.1	–	–	–	–	13	PED	NA	HA	NA	NA	c.388G > A p.Gly130Ser Wang et al. [25] Segregation not available

Pt patient, *F* female, *M* male, *NA* not available, *y* years, seizure type: *ABS* absence, *FS* febrile seizures, *GTC* generalized tonic–clonic, *MA* myoclonic–atonic, *MYO* myoclonic, *FM* focal motor, *FC* focal complex, *DR* drug resistance, EEG: *G* generalized discharges, *F* focal discharges, *SW* spike-and-wave complexes, movement disorder: *PED* paroxysmal exertion-induced dyskinesia, *PNKD* paroxysmal non-kinesigenic dyskinesia, *PKD* paroxysmal kinesigenic dyskinesia, *PD* paroxysmal dyskinesia. Neurological examination: *a* ataxia, *d* dystonia, *m* myoclonias, *DD* developmental delay, *ID* intellectual disability, *MC* microcephaly, *HA* headache, *Sp* spasticity, *SLD* Specific Learning Disabilities, *CSF* cerebrospinal fluid, *G* glucose, *L* lactate

carried out during the diagnostic work-up of different neurological syndromes (e.g. ataxia, dystonia).

Comparison between mutated and non-mutated patients

We compared the clinical pictures of the 19 *SLC2A1*-mutated patients from 18 families with those of the 226 non-mutated patients (Table 2). No statistically significant differences were found in the two groups with regard to gender, mean age at the time of genetic analysis, family history for epilepsy or movement disorders and developmental delay. At the time of genetic testing, there was a significantly high proportion of patients with acquired microcephaly in mutated patients (47.4% vs 13.7%, $p < 0.0001$; OR 5.6; 95% CI 2.1–15; $p = 0.001$).

Epilepsy was the most common symptom in both mutated (84.2%) and non-mutated patients (74.3%). Seizures were mostly generalized and were drug resistant in a similar proportion of patients in both groups. The mean age at onset of epilepsy in mutated patients was lower than in non-mutated (2.4 vs 4.9 years), although this difference did not reach a statistical significance. Nonetheless, there was a significantly different proportion of patients with onset of epilepsy in the first year of life: seizures started within the first year in 8/16 mutated patients and in 33/168 non-mutated patients (50% vs 19.6% $p < 0.005$; OR 21.8; 95% CI 6.0–79.3; $p < 0.001$).

Thirty-eight patients underwent molecular analysis in the diagnostic work-up for PD, either occurring as an isolated symptom or associated with epilepsy. Isolated PD, which had a similar age of onset in the two groups, was found in 15.7% of mutated patients and in 12.8% of non-mutated. In contrast, the association of epilepsy and PD was found in higher proportion among *SLC2A1*-mutated patients than in non-mutated (57.9% versus 3.98%, %, $p < 0.0001$; OR 33.1; 95% CI 10.7–102.5; $p < 0.001$).

Discussion

We report the analysis of the clinical and molecular finding in a large series of patients who underwent *SLC2A1* genetic screening.

As soon as our Lab was able to perform mutational analysis of *SLC2A1* gene, we received a large amount of DNA samples to be analyzed, both from our Institution and from other referring centers. It is likely that in a number of cases, the indication to the analysis was simply the search for a treatable disorder and in other the hope for a solution in unsolved and complex cases. This "enlarged indication" probably explains the low ratio (19/354) of confirmed diagnosis of a disease, that still remains extremely rare. Within this cohort, we identified 19 patients from 18 families carrying variants of pathological significance (previously described) or of possible pathological significance (novel variants). To define the indications for molecular analysis of the gene, we compared clinical features of mutated and non-mutated patients.

Patients carrying mutations

The analysis of the clinical features of the patients carrying *SLC2A1* gene variants confirms the wide clinical spectrum associated with Glut1 defects [17]. In our cohort, in line with previous reports, epilepsy was the most frequent presenting symptom. In a few patients, spontaneous seizures had been preceded by "febrile convulsions". This suggests that, beside fasting, fever might also be considered as a trigger factor for seizures, at least during infancy. Further unusual symptoms were fatigue- and food-related disorders, such as feeling hunger and compulsory eating (with ensuing overweight), which may mask the classical worsening of symptoms during fasting.

In our cohort, paroxysmal dyskinesia was present in 12/19 mutated patients, and, in line with literature data [17], featured as PED in most cases. A minority of patients, however, experienced PNKD and PKD. These latter cases, together with the few previously reported [18], widen the spectrum of paroxysmal dyskinesia in Glut1 DS, and confirm that all types of paroxysmal dyskinesia should be considered among the key symptoms of the disease. Paroxysmal dyskinesia appeared in 10 out of the 16 patients with epilepsy, during the follow-up (after a mean interval of 5.9 ± 2.2 years); whereas, it was the presenting symptom in 3 patients. Interestingly, in our series, when PD was the presenting symptom, its onset was in late childhood or adolescence and no seizure appeared during the prolonged follow-up.

Beside epilepsy and PD, which were the prominent symptoms, further paroxysmal symptoms varied according to the different ages. In infants, paroxysmal ocular movements might precede the onset of epilepsy; these abnormal movements had been frequently misdiagnosed as focal seizures. In contrast, in late childhood, adolescence and adulthood, headache was the most frequent paroxysmal symptom. Non-paroxysmal symptoms which included acquired microcephaly, intellectual disability of various degree, and motor deficits (ataxia, spasticity, dystonia) of variable severity were present in the majority of patients.

Comparing mutated and non-mutated patients

We compared the clinical features in the 19 mutated and in the 226 non-mutated patients. By the comparison between these two cohorts, it emerged that (1) the presence of epilepsy with onset within the first year of life, particularly when associated with developmental delay or other

Table 2 Comparison of clinical features in *SLC2A1*-mutated and non-mutated patients

	SLC2A1− (*n* = 226)	*SLC2A1*+ (*n* = 19)	X^2	OR, 95% CI, *p* value
a. Demographic data				
Gender				
Male	110 (48.7%)	7 (36.8%)	0.32	
Female	116 (51.3%)	12 (63.2%)		
Age at diagnosis (years) mean ± SD	12.5 ± 11.8	16.0 ± 7.6	0.21	
Family history of epilepsy or movement disorders				
Yes	78 (34.5%)	5 (26.3%)	0.46	
No	148 (65.4%)	14 (73.7%)		
b. Epilepsy				
Yes	168 (74.3%)	16 (84.2%)	0.50	
No	47 (20.7%)	3 (15.8%)		
Not known	11 (4.8%)	0 (0.0%)		
Onset of epilepsy years (mean ± SD)	4.9 ± 4.8	2.4 ± 2.8	0.04	
Onset of epilepsy < 12 months	33/168 (19.6%)	8/16 (50%)	0.005	OR 21.8 95% CI 6.0–79.3 *p* < 0.001
Drug resistant				
Yes	89 (53.0%)	6 (31.5%)	0.19	
No	57 (33.9%)	9 (47.3%)		
Not known	22 (13.1%)	1 (5.2%)		
c. Paroxysmal movement disorder (PMD)				
Yes	38 (16.8%)	13 (68.4%)	< 0.0001	OR 4.8 95% CI 2.1–10.8 *p* < 0.001
No	184 (81.4%)	6 (31.6%)		
Onset (years) mean ± SD	8.0 ± 8.2	8.4 ± 4.5	0.86	
d. Epilepsy and PMD				
Yes	9 (3.9%)	11 (57.9%)	< 0.0001	OR 33.1 95% CI 10.7–102.5 *p* < 0.001
No	217 (96.0%)	8 (42.1%)		
e. Other clinical features				
Developmental delay				
Yes	91 (40.2%)	6 (31.6%)	0.45	
No	135 (59.7%)	13 (68.4%)		
Microcephaly				
Yes	31 (13.7%)	9 (47.4%)	< 0.0001	OR 5.6 95% CI 2.1–15.0 *p* = 0.001
No	195 (86.2%)	10 (52.6%)		
Headache				
Yes	26 (11.5%)	4 (21%)	0.22	
No	200 (88.4%)	15 (78.9%)		
Neurological exam				
Normal	163 (72.1%)	9 (47.4%)	0.02	
Abnormal	63 (27.9%)	10 (52.6%)		

neurological manifestations, (2) the association of epilepsy with PD and (3) acquired microcephaly, is significantly more common in mutated patients. In fact, even if these three symptoms *per se* are not pathognomonic of the disease, their combined presence may suggest the diagnosis of Glut1 DS and rapidly lead to the procedures necessary to confirm the diagnostic suspect, including the genetic analysis of *SLC2A*1 gene.

Conclusions

The analysis of *SLC2A1* gene in a large series of patients referred to our Lab allowed to identify novel variants of possible pathological significance. The predictive characteristics (in silico analysis) of these novel variants and the segregation in the families of the probands support their causative role in the disease.

Glut1 deficiency has been reported to underlie genetic generalized epilepsies and, therefore, this diagnosis must be considered in all familial epilepsies [9, 19, 20].

However, in line with previous reports and reviews, our data confirm that Glut1 DS is a multi-symptomatic disorder, albeit with a wide spectrum of expressivity. In sporadic cases (which are the vast majority of the study cohort reported in this paper), Glut1 DS should be strongly suspected only in patients presenting with epilepsy associated with at least one of the following: microcephaly, movement disorders including all the three types of paroxysmal dyskinesia, psychomotor delay/intellectual disability, or one of the less common symptoms reported above. By contrast, Glut1 DS is extremely unlikely in patients featuring isolated epilepsy or isolated movement disorder [10, 15, 21].

Acknowledgements The authors are deeply grateful to: Drs Francesca Darra (Child Neuropsychiatry, University of Verona), Stefania Bergamoni (Azienda Ospedaliera Ospedale Niguarda Ca' Granda, Milano), Monica Lodi (Centro Regionale per l'Epilessia, Asst Fatebenefratelli Sacco, Milano) and Carmen Bonino (Torino) for referring patients and to Dr. Sara Matricardi (Department of Neuropsychiatry, Children's Hospital "G. Salesi", Ospedali Riuniti, Ancona) for her help with statistical analysis.

Author contributions Genetic screening and analysis, writing of the manuscript (BC, AV, CG); selection of patients, collection and analysis of clinical data (FR, EF, SC, RS, GT, BS, NN, TG); selection of patients, collection of clinical and EEG data (LC, FV, SF); clinical and genetic data analysis, writing of the manuscript (BC, FR, JCD); clinical and genetic data analysis, writing of the manuscript, coordination of the selection of patients, final supervision of the manuscript and submission (CG, TG).

Funding The present work was supported by the Bando Cariplo 2010 per la ricerca Biomedica no. 2010.0759, the Italian Ministry of Health Ricerca Finalizzata Giovani Ricercatori 2010 (Project GR-2010-2304834 to JCD) and Ricerca Finalizzata Giovani Ricercatori 2016 (Project GR-2016-02363337 to JCD), and Grants from the Pierfranco and Luisa Mariani Foundation (to TG).

Compliance with ethical standards

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI (1991) Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N Engl J Med 325(10):703–709
2. Seidner G, Alvarez MG, Yeh JI, O'Driscoll KR, Klepper J, Stump TS et al (1998) GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. Nat Genet 18(2):188–191. https://doi.org/10.1038/ng0298-188
3. Brockmann K (2009) The expanding phenotype of GLUT1 -deficiency syndrome. Brain Dev 7:545–552. https://doi.org/10.1016/j.braindev.2009.02.008
4. Weber YG, Storch A, Wuttke TV, Brockmann K, Kempfle J, Maljevic S et al (2008) GLUT1 mutations are a cause of paroxysmal exertion-induced dyskinesias and induce hemolytic anemia by a cation leak. J Clin Invest 118 6:2157–2168. https://doi.org/10.1172/JCI34438
5. Pong AW, Geary BR, Engelstad KM, Natarajan A, Yang H, De Vivo DC (2012) Glucose transporter type I deficiency syndrome: epilepsy phenotypes and outcomes. Epilepsia 53(9):1503–1510. https://doi.org/10.1111/j.1528-1167.2012.03592.x
6. Arsov T, Mullen SA, Damiano JA, Lawrence KM, Huh LL, Nolan M et al (2012) Early onset absence epilepsy: 1 in 10 cases is caused by GLUT1 deficiency. Epilepsia 53(12):e204–e207. https://doi.org/10.1111/epi.12007
7. Suls A, Mullen SA, Weber YG, Verhaert K, Ceulemans B, Guerrini R et al (2009) Early-onset absence epilepsy caused by mutations in the glucose transporter GLUT1. Ann Neurol 66 3:415–419. https://doi.org/10.1002/ana.21724
8. Mullen SA, Marini C, Suls A, Mei D, Della Giustina E, Buti D et al (2011) Glucose transporter 1 deficiency as a treatable cause of myoclonic astatic epilepsy. Arch Neurol 68 9:1152–1155. https://doi.org/10.1001/archneurol.2011.102
9. Arsov T, Mullen SA, Rogers S, Phillips AM, Lawrence KM, Damiano JA et al (2012) Glucose transporter 1 deficiency in the idiopathic generalized epilepsies. Ann Neurol 72 5:807–815. https://doi.org/10.1002/ana.23702
10. Agostinelli S, Traverso M, Accorsi P, Beccaria F, Belcastro V, Capovilla G et al (2013) Early-onset absence epilepsy: SLC2A1 gene analysis and treatment evolution. Eur J Neurol 20 5:856–859. https://doi.org/10.1111/j.1468-1331.2012.03871
11. Larsen J, Johannesen KM, Ek J, Tang S, Marini C, Blichfeldt S et al (2015) The role of SLC2A1 mutations in myoclonic astatic epilepsy and absence epilepsy, and the estimated frequency of GLUT1 deficiency syndrome. Epilepsia 56(12):e203–e208. https://doi.org/10.1111/epi.13222
12. Leen WG, Wevers RA, Kamsteeg EJ, Scheffer H, Verbeek MM, Willemsen MA (2013) Cerebrospinal fluid analysis in the workup of GLUT1 deficiency syndrome: a systematic review. JAMA Neurol 70 11:1440–1444. https://doi.org/10.1001/jamaneurol.2013.3090
13. Klepper J, Scheffer H, Leiendecker B, Gertsen E, Binder S, Leferink M et al (2005) Seizure control and acceptance of the ketogenic diet in GLUT1 deficiency syndrome: a 2- to 5-year follow-up of 15 children enrolled prospectively. Neuropediatrics 36 5:302–308. https://doi.org/10.1055/s-2005-872843
14. Kass HR, Winesett SP, Bessone SK, Turner Z, Kossoff EH (2016) Use of dietary therapies amongst patients with GLUT1 deficiency syndrome. Seizure 35:83–87. https://doi.org/10.1016/j.seizure.2016.01.011
15. Ragona F, Matricardi S, Castellotti B, Patrini M, Freri E, Binelli S et al (2014) Refractory absence epilepsy and Glut1 deficiency syndrome: a new case report and literature review. Neuropediatrics 45 5:328–332. https://doi.org/10.1055/s-0034-1378130

16. Zorzi G, Castellotti B, Zibordi F, Gellera C, Nardocci N (2008) Paroxysmal movement disorders in GLUT1 deficiency syndrome. Neurology 71 2:146–148. https://doi.org/10.1212/01.wnl.0000316804.10020
17. Koch H, Weber YG (2018) The glucose transporter type 1 (Glut1) syndromes. Epilepsy Behav. https://doi.org/10.1016/j.yebeh.2018.06.010
18. De Giorgis V, Varesio C, Baldassari C, Piazza E, Olivotto S, Macasaet J et al (2016) Atypical manifestations in Glut1 deficiency syndrome. J Child Neurol 31 9:1174–1180. https://doi.org/10.1177/0883073816650033
19. Mullen SA, Suls A, De Jonghe P, Berkovic SF, Scheffer IE (2010) Absence epilepsies with widely variable onset are a key feature of familial GLUT1 deficiency. Neurology 75(5):432–440. https://doi.org/10.1212/WNL.0b013e3181eb58b4
20. Striano P, Weber YG, Toliat MR, Schubert J, Leu C, Chaimana R, Baulac S, Guerrero R, LeGuern E, Lehesjoki AE, Polvi A, Robbiano A, Serratosa JM, Guerrini R, Nürnberg P, Sander T, Zara F, Lerche H, Marini C; EPICURE Consortium (2012) GLUT1 mutations are a rare cause of familial idiopathic generalized epilepsy. Neurology 78(8):557–562. https://doi.org/10.1212/WNL.0b013e318247ff54
21. Verrotti A, D'Egidio C, Agostinelli S, Gobbi G (2012) Glut1 deficiency: when to suspect and how to diagnose? Eur J Paediatr Neurol 16(1):3–9. https://doi.org/10.1016/j.ejpn.2011.09.005 **(Epub 2011 Oct 1. Review. PMID: 21962875)**
22. Urbizu A, Cuenca-León E, Raspall-Chaure M, Gratacòs M, Conill J, Redecillas S, Roig-Quilis M, Macaya A (2010) Paroxysmal exercise-induced dyskinesia, writer's cramp, migraine with aura and absence epilepsy in twin brothers with a novel SLC2A1 missense mutation. J Neurol Sci 295(1–2):110–113. https://doi.org/10.1016/j.jns.2010.05.017
23. Liu Y, Bao X, Wang D, Fu N, Zhang X, Cao G, Song F, Wang S, Zhang Y, Qin J, Yang H, Engelstad K, De Vivo DC, Wu X (2012) Allelic variations of glut-1 deficiency syndrome: the chinese experience. Pediatr Neurol 47(1):30–34. https://doi.org/10.1016/j.pediatrneurol.2012.04.010
24. Schneider SA, Paisan-Ruiz C, Garcia-Gorostiaga I, Quinn NP, Weber YG, Lerche H, Hardy J, Bhatia KP (2009) GLUT1 gene mutations cause sporadic paroxysmal exercise-induced dyskinesias. Mov Disord 24(11):1684–1688. https://doi.org/10.1002/mds.22507
25. Wang D, Pascual JM, Yang H, Engelstad K, Jhung S, Sun RP, De Vivo DC (2005) Glut-1 deficiency syndrome: clinical, genetic, and therapeutic aspects. Ann Neurol 57(1):111–118. https://doi.org/10.1002/ana.20331