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SHORT REPORT

The clinical and biochemical hallmarks generally associated with GLUT1DS may be caused by defects in genes other than *SLC2A1*

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Abstract

Glucose transporter 1 deficiency syndrome (GLUT1DS) is a neurometabolic disorder caused by haploinsufficiency of the GLUT1 glucose transporter (encoded by *SLC2A1*) leading to defective glucose transport across the blood–brain barrier. This work describes the genetic analysis of 56 patients with clinical or biochemical GLUT1DS hallmarks. 55.4% of these patients had a pathogenic variant of *SLC2A1*, and 23.2% had a variant in one of 13 different genes. No pathogenic variant was identified for the remaining patients. Expression analysis of *SLC2A1* indicated a reduction in *SLC2A1* mRNA in patients with pathogenic variants of this gene, as well as in one patient with a pathogenic variant in *SLC9A6*, and in three for whom no candidate variant was identified. Thus, the clinical and biochemical hallmarks generally associated with GLUT1DS may be caused by defects in genes other than *SLC2A1*.

Rafael Artuch and Belén Pérez are joint senior authors.

KEYWORDS
GLUT1, GLUT1DS, hypoglycorrhachia, *SLC2A1*

1 | INTRODUCTION

Glucose transporter 1 deficiency syndrome (GLUT1DS, MIM: #606777) is a neurometabolic disorder caused by haploinsufficiency of the GLUT1 glucose transporter leading to defective glucose transport across the blood–brain barrier. In general, this syndrome is an autosomal dominant disorder caused by heterozygous pathogenic variants (de novo or inherited) of *SLC2A1* (MIM: *138140), although some patients showing autosomal recessive inheritance have been reported.[1,2]

The main biochemical marker of GLUT1DS is hypoglycorrhachia. Patients with classic disease also have drug-refractory epilepsy *HP:0001250*, show developmental delay *HP:0001263*, complex movement disorders *HP:0100022* (spasticity *HP:0001257*, ataxia *HP:0001251* and dystonia *HP:0001332*), and acquired microcephaly *HP:0005484* (50% of cases).[1,3] However, a broader phenotypic spectrum is recognised.[4]

Patients respond to a ketogenic diet with improvements in seizure frequency and intensity, and associated complex movement disorders.[5,6] Nonetheless, these diets are not problem-free and patients can run out of options.[5,6]

The aim of this work was to determine the genetic basis of suspected GLUT1DS in patients with clinical or biochemical signs of GLUT1DS. Interestingly, variants in genes other than *SLC2A1* were found that would appear to give rise to the same hallmark clinical and biochemical signs of this disease.

2 | MATERIALS AND METHODS

The study subjects were 56 patients from 54 families (P25 and P26 and also P48 and P49 are siblings); all had been referred to our facility from different neurological units in Spain for genetic confirmation of suspected GLUT1DS. All had either a low-CSF glucose (<50.5 mg/dl) plus a low-CSF/blood glucose ratio (≤0.65) in the presence of low to normal lactate values (we decided to broaden the CSF/blood glucose ratio[7] as it has been described in 2013),[8] clinical findings suggestive of GLUT1DS (seizures, developmental delay, movement disorders [persistent or paroxysmal] and/or acquired microcephaly) or both. Clinical symptoms and biochemical data were annotated using Human Phenotype Ontology (HPO) terms (https://hpo.jax.org/).[9] The present study was approved by the Ethics Committee of the *Universidad Autónoma de Madrid* on February 19, 2018 (CEI-85-1594).

To identify the variants giving rise to the above clinical and biochemical findings, the exonic or entire sequence of *SLC2A1* (included the intronic sequences) was analysed by Sanger sequencing or next generation sequencing, respectively. To detect changes in the methylation of the *SLC2A1* canonical CpG island, sodium bisulphite modification was performed using the EZ DNA Methylation-Gold Kit (Zymo Research). Methylation-specific PCR (MSP) was then performed under standard PCR conditions.

When no pathogenic variant of *SLC2A1* was found, patient DNA was sequenced using the Clinical-Exome Sequencing (CES) TruSight™ One Gene Panel and/or the Whole Exome Sequencing (WES) TruSeq Exome Kit (Illumina).

SLC2A1 mRNA was quantified by RT-qPCR analyses of fibroblasts derived from healthy controls ($n = 2$) and patients ($n = 19$) using a LightCycler® 480 instrument (Roche Applied Science), the NZY First-Strand cDNA Synthesis Kit (NZYTech), and the PerfeCTa SYBR® Green FastMix Kit (Quantabio). *GUSB* was used as an endogenous control.

Data with non-normal distributions were Log_2 transformed before analysis. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used for multiple comparisons between groups.

3 | RESULTS

Forty (71.4%) of the present patients had suffered some type of seizure, 31 (55.4%) had some degree of neurodevelopmental delay, 40 (71.4%) had movement disorder symptoms, and 11 (19.6%) had microcephaly (Tables 1 and 2 and Table S1).

Pathogenic *SLC2A1* variants were found in 31 patients (55.4%). The mutational spectrum of *SLC2A1* included two large deletions, four small deletions, two small duplications, one variant in a regulatory region (5′UTR), and 20 nucleotide changes (17 likely missense [Table S2], one nonsense, and two splice site variants). Fifteen variants were novel (Table 1). No abnormalities in *SLC2A1* methylation were found.

Among 13 patients with no pathogenic variants of *SLC2A1*, 11 of whom had hypoglycorrhachia, pathogenic or likely pathogenic variants were found in 13 different genes. All these genes have described variants or have intolerant pLI and O/E scores (Table 2; Table S3 lists the HPOs terms relating to *SLC2A1* and these genes). The presence of hypoglycorrhachia suggests that *SLC2A1* expression might be altered in these 13 patients as an effect of variation in these other genes. RT-qPCR revealed a significant reduction in *SLC2A1* mRNA expression in patient P34-derived fibroblasts compared to healthy controls (Figure 1). This suggests that the variant in *SLC9A6* carried by this patient might cause secondary *SLC2A1* haploinsufficiency. RT-qPCR analysis also showed a meaningful reduction in *SLC2A1* mRNA expression in fibroblasts from patients P48, P49 and P52 (Figure 1). This might be secondary to non-*SLC2A1* gene defects carried by them

TABLE 1 Genotype and phenotype of *SLC2A1* cases

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Variants	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
P1	11 y	1 y	32	0.4	9	g.42477481_44170170del	*De novo*	New	Pathogenic	Global developmental delay HP:0001263, microcephaly HP:0000252, seizure HP:0001250, hypoglycorrhachia HP:0011972
P2	17 y	No data	37	0.41	1.6	c.505_507delp.(Leu169del)	*De novo*	CD044162	Pathogenic	Atonic seizures HP:0010819, exercise-induced muscle fatigue HP:0009020, EEG abnormality HP:0002353, hypoglycorrhachia HP:0011972
P3	27 y	17 y	38	0.4	11.3	c.823G>Ap.(Ala275Thr)	Maternal	CM081810	Pathogenic	Paroxysmal dyskinesia HP:0007166 (Induced by exercise), hypoglycorrhachia HP:0011972
P4	19 y	No data	40	0.39	Normal	c.711_712delp.(Thr238Profs*2)	*De novo*	New	Pathogenic	Ataxia HP:0001251, hypotonia HP:0001252, exercise-induced muscle fatigue HP:0009020, seizure HP:0001250, hypoglycorrhachia HP:0011972
P5	14 y	7 y	42	0.46	9	c.1232A>Gp.(Asn411Ser)	Maternal	CM1212157	Pathogenic	Early onset absence seizures HP:0011152, hypoglycorrhachia HP:0011972
P6	22 y	No data	30	0.33	7.5	g.43307942_43437676del	Not done	New	Pathogenic	Global developmental delay HP:0001263, seizure HP:0001250, ataxia HP:0001251, abnormality of extrapyramidal motor function HP:0002071, hypotonia HP:0001252, microcephaly HP:0000252, myoclonus HP:0001336, hypoglycorrhachia HP:0011972
P7	27 y	No data	40	0.49	No data	c.1202C>Gp.(Pro401Arg)	Not done	New	Likely pathogeni	Global developmental delay HP:0001263, seizure HP:0001250, dystonia HP:0001332, hypoglycorrhachia HP:0011972
P8	13 y	No data	28	0.35	No data	c.1097_1100delp.(Tyr366*)	*De novo*	CD1918695	Pathogenic	Focal impaired awareness seizure HP:0002384, clumsiness HP:0002312, tremor HP:0001337, ataxia HP:0001251, cognitive impairment HP:0100543, global developmental delay HP:0001263, hypoglycorrhachia HP:0011972
P9	10 y	No data	38	0.42	No data	c.103G>Ap.(Ala35Thr)	Not done	New	Likely pathogenic	Global developmental delay HP:0001263, dystonia HP:0001332, generalized myoclonic seizure HP:0002123, clumsiness HP:0002312, hypoglycorrhachia HP:0011972
P10	3 y	5 m	31	0.32	11	c.-107G>A p.?	*De novo*	CR177206	Pathogenic	Global developmental delay HP:0001263, microcephaly HP:0000252, abnormality of eye movement HP:0000496, esodeviation HP:0020045, paroxysmal involuntary eye movements HP:0007704, abnormal head movements HP:0002457, hypoglycorrhachia HP:0011972 Video: abnormality of eye movement HP:0000496 and abnormal head movements HP:0002457
P11	27 y	13 y	32	0.38	7	c.524G>Tp.(Gly175Val)	Not done	New	Likely pathogenic	Seizure HP:0001250, cognitive impairment HP:0100543, scoliosis HP:0002650, hypoglycorrhachia HP:0011972, abnormal cerebellum morphology HP:0001317

TABLE 1 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Variants	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
P12	8 y	3 y	32	No data	No data	c.485T>G p.(Leu162Arg)	Not done	New	Likely pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, abnormality of eye movement HP:0000496, oculogyric crisis HP:0010553, ataxia HP:0001251, abnormality of extrapyramidal motor function HP:0002071, short attention span HP:0000736
P13	24 y	13 y	34	0.39	10	c.18+2T>G p.?	*De novo*	CS1411096	Pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, seizure HP:0001250, early onset absence seizures HP:0011152, cognitive impairment HP:0100543, secondary microcephaly HP:0005484, ataxia HP:0001251, abnormality of extrapyramidal motor function HP:0002071, abnormal pyramidal sign HP:0007256, hypoglycorrhachia HP:0011972
P14	37 y	20 y	38	0.39	13	c.1346_1359del p.(Tyr449*)	Not done	CD101727	Pathogenic	Epileptic spasms HP:0011097, generalized-onset seizure HP:0002197, focal-onset seizure HP:0007359, generalized non-motor (absence) seizure HP:0002121, myoclonic spasms HP:0003739, cognitive impairment HP:0100543, truncal ataxia HP:0002078, hyperreflexia HP:0001347, clumsiness HP:0002312, speech apraxia HP:0011098, hypoglycorrhachia HP:0011972
P15	22 y	11 y	40	0.42	10	c.998G>A p.(Arg333Gln)	Paternal	CM095401	Pathogenic	Delayed speech and language development HP:0000750, seizure HP:0001250, atypical absence seizure HP:0007270, dystonia HP:0001332, dysarthria HP:0001260, hypoglycorrhachia HP:0011972
P16	13 y	8 y	41	0.5	No data	c.140C>T p.(Thr47Ile)	Maternal	New	VUS	Seizure HP:0001250, atypical absence seizure HP:0007270, episodic ataxia HP:0002131, paroxysmal dyskinesia HP:0007166, hypoglycorrhachia HP:0011972
P17	8 y	2 y	25	0.27	No data	c.1265dup p.(Gln423Profs*32)	*De novo*	New	Pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, cognitive impairment HP:0100543, ataxia HP:0001251, dyskinesia HP:0100660, hypoglycorrhachia HP:0011972
P18	11 y	5 y	32	0.32	9.1	c.805C>T p.(Arg269Cys)	Maternal	CM135625	Likely pathogenic	Clinical symptoms compatible with Rett syndrome
P19	11 y	6 y	39.6	0.41	No data	c.1114A>T p.(Ile372Phe)	*De novo*	New	Likely pathogenic	Triggered by fasting HP:0025212, paroxysmal dyskinesia HP:0007166, clumsiness HP:0002312, specific learning disability HP:0001328, hypoglycorrhachia HP:0011972
P20	12 y	7 y	35	0.39	No data	c.64G>C p.(Gly22Arg)	*De novo*	New	Likely pathogenic	Paroxysmal dyskinesia HP:0007166 (Induced by exercise), Global developmental delay HP:0001263, Hypoglycorrhachia HP:0011972

(Continues)

TABLE 1 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Variants	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
P21	6 y	4 m	42	0.38	8.7	c.1387A>C/c.1387A>Cp.(Ile463Leu)/p.(Ile463Leu)	Not done	New	VUS	Hypoglycorrhachia HP:0011972
P22	26 y	6 y	27	0.22	8.2	c.101A>G p.(Asn34Ser)	Not done	CM052363	Pathogenic	Hypoglycorrhachia HP:0011972
P23	7 y	3 y	No data	No data	No data	c.1453C>A p.(Pro485Thr)	Not done	New	Likely pathogenic	Global developmental delay HP:0001263, cognitive impairment HP:0100543, autistic behaviour HP:0000729, abnormal facial shape HP:0001999
P24	11 y	4 y	No data	No data	No data	c.971C>T p.(Ser324Leu)	Not done	CM096019	Pathogenic	Dystonia HP:0001332, appendicular hypotonia HP:0012389, EEG abnormality HP:0002353
P25[d]	21 y	17 y	No data	No data	No data	c.632C>A p.(Pro211His)	Not done	New	Likely pathogenic	Cognitive impairment HP:0100543, seizure HP:0001250, early onset absence seizures HP:0011152, clumsiness HP:0002312, behavioural abnormality HP:0000708
P26[d]	23 y	22 y	No data	No data	No data	c.632C>A p.(Pro211His)	Not done	New	Likely pathogenic	Global developmental delay HP:0001263, cognitive impairment HP:0100543, seizure HP:0001250, early onset absence seizures HP:0011152, clumsiness HP:0002312, tremor HP:0001337
P27	7 y	2 y	29	0.3	No data	c.680-1G>Cp.?	Not done	CS057229	Pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, abnormality of eye movement HP:0000496, atypical absence seizure HP:0007270, hypoglycorrhachia HP:0011972
P28	21 y	5 y	34	0.4	6	c.1057_1058dup p.(Ala354Serfs*3)	Not done	New	Pathogenic	Global developmental delay HP:0001263, cognitive impairment HP:0100543, generalized non-motor (absence) seizure HP:0002121, early onset absence seizures HP:0011152, seizure HP:0001250, paroxysmal dyskinesia HP:0007166, myoclonus HP:0001336, ataxia HP:0001251, abnormal pyramidal sign HP:0007256, abnormality of extrapyramidal motor function HP:0002071, dystonia HP:0001332, dysarthria HP:0001260, hypoglycorrhachia HP:0011972
P29	10 y	8 y	29	0.36	No data	c.457C>T p.(Arg153Cys)	Maternal	CM044066	Likely pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, seizure HP:0001250, generalized non-motor (absence) seizure HP:0002121, generalized myoclonic seizures HP:0002123, clumsiness HP:0002312, hypoglycorrhachia HP:0011972
P30	14 y	11 y	34	No data	10	c.457C>T p.(Arg153Cys)	Maternal	CM044066	Likely pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, cognitive impairment HP:0100543, seizure HP:0001250, generalized myoclonic seizures HP:0002123, paroxysmal dyskinesia HP:0007166, impaired executive functioning HP:0033051, abnormal social behaviour HP:0012433

TABLE 1 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Variants	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
P31	17 y	14 y	No data	No data	No data	c.748C>T p.(Gln250*)	Not done	CM1820795	Pathogenic	Global developmental delay HP:0001263, delayed speech and language development HP:0000750, cognitive impairment HP:0100543, seizure HP:0001250, bilateral tonic-clonic seizure HP:0002069, generalized myoclonic seizures HP:0002123, abnormal pyramidal sign HP:0007256, tetraparesis HP:0002273, dystonia HP:0001332

Note: Genome reference hg19/GRCh37. The genomic reference sequence used was NC_000001.10 and the coding DNA reference sequence used was NM_006516.4. HGVS guidelines were used for variant description. Accession number from HGMD® Professional 2019.2 (https://portal.biobase-international.com/hgmd/pro/start.php?) are included. Human Phenotype Ontology terms were obtained from the HPO website (https://hpo.jax.org/app/).

Abbreviations: ACMG, American College of Medical Genetics and Genomics; CSF, cerebrospinal fluid; HGMD, Human Gene Mutation Database; m, months; VUS, variant of uncertain significance; y, years.

[a]Age at which lumbar puncture was performed.

[b]Ratio: cerebrospinal fluid to serum blood glucose.

[c]The variants identified were classified in five categories (benign, likely benign, variant of unknown significance (VUS), likely pathogenic, and pathogenic) according to ACMG guidelines using the VarSome web platform (https://varsome.com/).

[d]Patient 25 and patient 26 are siblings.

or to variants in *SLC2A1* not detectable by the technology employed.

4 | DISCUSSION

In the present work, variants in *SLC2A1* were found in only 55.4% (31/56) of the examined patients, a low figure compared to other European series.[10] Agnostic analysis solved 13 additional cases, increasing the diagnosis rate to nearly the 80%. In these patients, pathogenic variants were identified in genes other than *SLC2A1* coding, for different ion channels, transporters, transcriptional factors, enzymes and receptors.

The present patients shared many clinical or biochemical features, including developmental delay, seizures, dystonia, microcephaly, ataxia and dyskinesia, etc., caused either by variants in *SLC2A1* or other genes. Among the 13 patients with variants in these other genes (i.e., not *SLC2A1*), 11 had hypoglycorrhachia. Until now, hypoglycorrhachia has only ever been reported in patients with defects in *SLC2A1*; thus, the variants of the other genes found to be involved might cause GLUT1DS via other mechanisms (something already reported for a *PURA* pathogenic variant).[11] Certainly, the present results show *SLC2A1* mRNA levels to be downregulated in fibroblasts from patients with genetic variations in *SLC9A6*, as well as in those from three patients (P48, P49 and P52) in whom no pathogenic variant could be identified in any gene. While this might account for hypoglycorrhachia in these few patients, the presence of this symptom in the other 13 patients with no *SLC2A1* defect suggests that low-CSF glucose is not a specific pathognomonic biomarker of defects in *SLC2A1*. It should be added that the pathogenic variant found in *SLC9A6* in patient P34 might affect the recycling pathway of several proteins, including GLUT1.[12] RNA-Seq in combination with whole genome sequencing (using short-read or long-read technologies) might help improve our understanding in this respect.[13–15]

HPO terms are very useful for harmonising clinical features, in delineating longitudinal disease phenotypes, and in integrating phenotypic data into diagnostic workflows.[16] However, and despite the important overlap between the HPOs of GLUT1DS associated with *SLC2A1* pathogenic variants and variants in the other genes here described, those associated with the former are rather distinct. For example, exercise-induced paroxysmal dyskinesia, fasting gait dyspraxia, and an excellent response of epileptic symptoms to a ketogenic diet, are suggestive of a *SLC2A1* defect.[4] Moreover, intellectual disability tends to be absent to mild–moderate in most patients with a *SLC2A1* defect. In the present cohort, however, those patients with defects in non-*SLC2A1* genes usually suffered from developmental encephalopathies with severe cognitive impairment. In fact, most of these other genes are involved in synaptic function. Since synaptic function and channel activity account for most of the energy consumed in the brain,[17] glucose homeostasis might be impaired if energy consumption is dysregulated. Therefore, the hypoglycorrhachia suffered by these

TABLE 2 Genotype and phenotype of patients with suspected GLUT1DS with variants in other genes

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
P32	6 y	1 y	44	0.55	9.1	*SCN8A* (NM_014191.4)	c.5267T>G p.(Ile1756Ser)	AD	1	0.06	*De novo*	New	Pathogenic	EEG abnormality HP:0002353, febrile seizure (within the age range of 3 months to 6 years) HP:0002373, Focal-onset seizure HP:0007359, Focal myoclonic seizures HP:0011166, abnormal involuntary eye movements HP:0012547, involuntary movements HP:0004305, tip-toe gait HP:0030051, hypoglycorrhachia HP:0011972
P33	15 y	9 y	47	0.55	Normal	*SETD1B* (NM_001353345.2)	c.697dup p.(Ser233Phefs*15)	AD	1	0.07	Not done	New	Pathogenic	Generalized non-motor (absence) seizure HP:0002121, myoclonic absence seizure HP:0011150, myoclonus HP:0001336, Sleep myoclonus HP:0012323, action tremor HP:0002345, postural tremor HP:0002174, motor delay HP:0001270, incoordination HP:0002311, cognitive impairment HP:0100543, short attention span HP:0000736,

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
														restlessness HP:0000711, impulsivity HP:0100710, impaired social reciprocity HP:0012760, EEG abnormality HP:0002353, hypoglycorrhachia HP:0011972
P34	17 y	8 y	46	0.48	10.0	*SLC9A6* (NM_006359.3)	c.803+1G>A p.(Val233Alafs*3)	X-LR			Maternal	CS1918586	Pathogenic	Severe global developmental delay HP:0011344, atypical absence seizures HP:0007270, bilateral tonic-clonic seizure HP:0002069, microcephaly HP:0000252 Gait ataxia HP:0002066, EEG abnormality HP:0002353, delayed speech and language development HP:0000750, Intellectual disability, severe HP:0010864, hypoglycorrhachia HP:0011972
P35	17 y	7 y	46	0.49	10.0	*NKX2-1* (NM_001079668.3)	c.727del p.(Arg243Alafs*4)	AD	0.36	0.23	Not done	CD1918589	Pathogenic	Tremor HP:0001337, postural tremor HP:0002174, abnormal brain positron emission tomography HP:0012657, specific learning disability HP:0001328, dysgraphia HP:0010526, Short attention span

(Continues)

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
														HP:0000736, hypothyroidism HP:0000821,poor fine motor coordination HP:0007010, chorea HP:0002072, dystonia HP:0001332, hypoglycorrhachia HP:0011972
P36	6 y	1 y	47 46	0.49 0.55	15.3 13.7	*ATP1A3* (NM_152296.5)	c.2401G>A p.(Asp801Asn)	AD	1	0	*De novo*	CM127591	Pathogenic	Widened subarachnoid space HP:0012704, abnormal cerebral ventricle morphology HP:0002118, hypoplasia of the corpus callosum HP:0002079, generalized hypotonia HP:0001290, pulmonary arterial hypertension HP:0002092, Left ventricular hypertrophy HP:0001712, focal-onset seizure HP:0007359, abnormal ascending aorta morphology HP:0031784, hypoglycorrhachia HP:0011972
P37	10 y	3 m	40 45	0.46 0.47	7.9 12.7	*KCNQ2* (NM_172107.4)	c.619C>T p.(Arg207Trp)	AD	1	0.05	*De novo*	CM014798	Pathogenic	Seizure HP:0001250, intellectual disability, moderate HP:0002342, behavioural abnormality HP:0000708, autistic behaviour

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
														HP:0000729, hypoglycorrhachia HP:0011972
P38	11 y	No data	46	0.60	10.0	*SLC6A1* (NM_003042.4)	c.278_279del p.(Ala93Glyfs*113)	AD	1	0.03	*De novo*	CD1918588	Pathogenic	Atypical absence seizures HP:0007270, hypermetropia HP:0000540, delayed speech and language development HP:0000750, global developmental delay HP:0001263, short attention span HP:0000736, hyporeflexia HP:0001265, EEG abnormality HP:0002353, behavioral abnormality HP:0000708, hypoglycorrhachia HP:0011972
P39	15 y	No data	46	0.56	No data	*NALCN* (NM_052867.4)	c.965T>C p.(Ile322Thr)	AD	0	0.4	*De novo*	CM1611146	Pathogenic	EEG abnormality HP:0002353, apnea HP:0002104, short stature HP:0004322, decreased body weight HP:0004325, episodic ataxia HP:0002131, dystonia HP:0001332, hypotonia HP:0001252, paroxysmal dyskinesia HP:0007166, hypermetropia HP:0000540, astigmatism HP:0000483,

(Continues)

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
														intellectual disability severe HP:0010864, clinodactyly HP:0030084, drooling HP:0002307, abnormality of the face HP:0000271, episodic fatigue HP:0012431, Delayed speech and language development HP:0000750, hypoglycorrhachia HP:0011972
P40	13 y	No data	49	0.63	No data	*CSNK2B* (NM_001320.7)	c.62del p.(Phe21Serfs*30)	AD	0.92	0.08	*De novo*	New	Pathogenic	Generalized non-motor (absence) seizure HP:0002121, bilateral tonic-clonic seizure HP:0002069, myoclonus HP:0001336, bruxism HP:0003763, global developmental delay HP:0001263, EEG abnormality HP:0002353, hypoglycorrhachia HP:0011972
P41	26 y	No data	No data	No data	No data	*DNM1* (NM_004408.4)	c.534C>G p.(Asn178Lys)	AD	1	0.13	Not done	New	Likely pathogenic	Encephalopathy HP:0001298, intellectual disability HP:0001249, abnormality of coordination HP:0011443, involuntary movements HP:0004305, paroxysmal dyskinesia HP:0007166

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
P42	7 y	1y	20	0.36	12.6	*MAN2B2* (NM_015274.3)	c.2912C>T/ c.2912C>T p.(Thr971Met)/ p.(Thr971Met)	AR			Paternal/ Maternal	New	Likely benign	Global developmental delay HP:0001263, hypotonia HP:0001252, Fatigue HP:0012378, microcephaly HP:0000252, delayed gross motor development HP:0002194, delayed speech and language development HP:0000750, failure to thrive HP:0001508, reduced consciousness/ confusion HP:0004372, action tremor HP:0002345, abnormality of coordination HP:0011443, motor delay HP:0001270, ataxia HP:0001251, dysmetria HP:0001310, broad-based gait HP:0002136, Joint laxity HP:0001388, muscle weakness HP:0001324, genu recurvatum HP:0002816, echolalia HP:0010529, bradykinesia HP:0002067, hyperlordosis

(Continues)

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
														HP:0003307, abnormal reflex HP:0031826, hypoglycorrhachia HP:0011972
P43	29 y	No data	No data	No data	No data	*NEXMIF* (NM_001008537.3)	c.1882C>T p.(Arg628*)	X-LD			Not done	CM140386	Pathogenic	Seizure HP:0001250, generalized-onset seizure HP:0002197, generalized non-motor (absence) seizure HP:0002121, eyelid myoclonia seizure HP:0032678, bilateral tonic-clonic seizure HP:0002069, EEG abnormality HP:0002353, intellectual disability HP:0001249, impairment of activities of daily living HP:0031058
P44	9 y	2 y	44	0.5	11.5	*UNC13A* (NM_001080421.2)	c.2422G>A p.(Gly808Ser)	AD	1	0.09	Not done	New	Uncertain significance	Seizure HP:0001250, Febrile seizure (within the age range of 3 months to 6 years) HP:0002373, Global developmental delay HP:0001263, Head tremor HP:0002346, Limb tremor HP:0200085, Action tremor HP:0002345, Stereotypical body rocking HP:0012172,

TABLE 2 (Continued)

REF.	Current age	Age at biochemical diagnostic[a]	CSF glucose (mg/dl)	Ratio[b]	CSF lactate (mg/dl)	Gene	Variants	Inheritancepattern	pLI	O/E	Inheritance	HGMD	ACMG[c]	Clinical data human phenotype ontology (HPO)
														Microcephaly HP:0000252, Dystonia HP:0001332, Sleep disturbance HP:0002360, Irritability HP:0000737, Hypoglycorrhachia HP:0011972

Note: Genome reference hg19/GRCh37. HGVS guidelines were used for variant description. pLI and O/E scores for variants with an AD inheritance pattern are displayed. These scores were obtained from gnomAD website (https://gnomad.broadinstitute.org/). Accession number from HGMD® Professional 2019.2 (https://portal.biobase-international.com/hgmd/pro/start.php) are included. Human Phenotype Ontology terms were obtained from the HPO website (https://hpo.jax.org/app/).

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; CSF, cerebrospinal fluid; HGMD, Human Gene Mutation Database; M, months; O/E, observed/expected; pLI, probability of being loss-of-function intolerant; X-LD, X-linked dominant; X-LR, X-linked recessive; Y, years.

[a]Age at which the first lumbar puncture was performed.

[b]Ratio: cerebrospinal fluid to serum blood glucose.

[c]The variants identified were classified in five categories (benign, likely benign, variant of unknown significance (VUS), likely pathogenic, and pathogenic) according to ACMG guidelines using the VarSome web platform (https://varsome.com/).

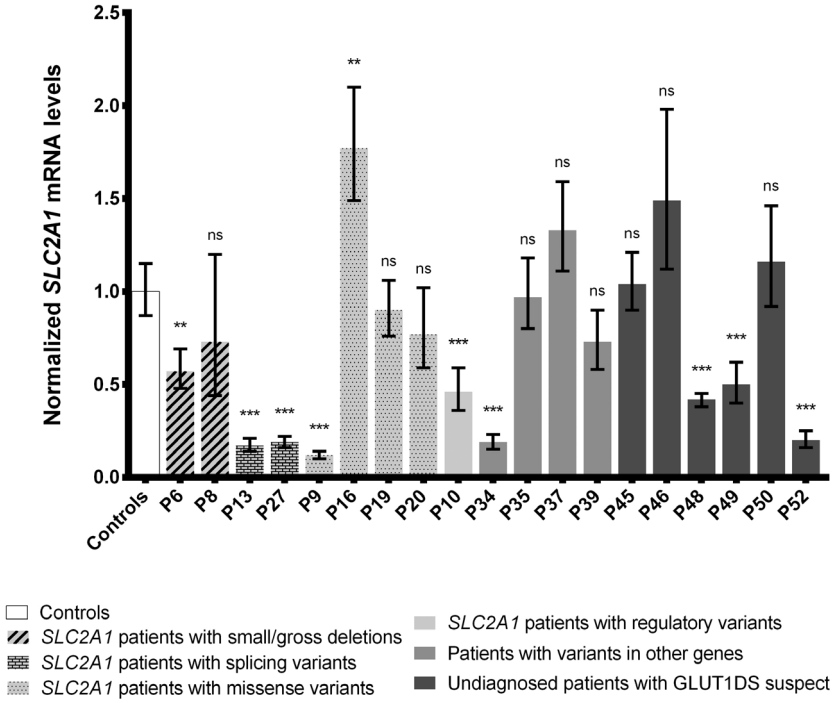

FIGURE 1 Expression of *SLC2A1* mRNA in patient-derived fibroblasts. Relative mRNA expression levels of *SLC2A1* in two healthy human fibroblast cell lines (controls), in the fibroblasts of nine patients with pathogenic variants in *SLC2A1* (P6, P8, P13, P27, P9, P16, P19, P20 and P10), four with suspected GLUT1DS and pathogenic variants in other genes (P34, P35, P37 and P39), and six patients with suspected GLUT1DS for whom no pathogenic variant was identified (P45, P46, P48, P49, P50 and P52). Data are represented as the mean ± SD of three experiments (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). *SLC2A1* mRNA levels were normalised using *GUSB* as an endogenous control

patients could be more an occasional finding than a biochemical signature of the affected non-*SLC2A1* genes.

In summary, the present results suggest that the clinical and biochemical hallmarks generally associated with GLUT1DS may be caused by defects in genes other than *SLC2A1*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings reported here are available from the corresponding authors upon reasonable request.

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SUPPORTING INFORMATION

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