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.
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 ratio7 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 Log2 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.
| REF. | Current age | Age at biochemical diagnostic | CSF glucose (mg/dl) | Ratio | CSF lactate (mg/dl) | Variants | Inheritance | HGMD | ACMG | 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, hypoglycorrachia HP:0011972 |
| P2   | 17 y        | No data                     | 37                  | 0.41  | 1.6               | c.505_507del.(Leu169del) | De novo   | CD044162 | Pathogenic | Atonic seizures HP:0010819, exercise-induced muscle fatigue HP:0009020, EEG abnormality HP:0002353, hypoglycorrachia 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), hypoglycorrachia HP:0011972 |
| P4   | 19 y        | No data                     | 40                  | 0.39  | Normal            | c.711_712delp. (Thr238Profs*2) | De novo   | New  | Pathogenic | Ataxia HP:0001251, hypertonia HP:0001252, exercise-induced muscle fatigue HP:0009020, seizure HP:0001250, hypoglycorrachia HP:0011972 |
| P5   | 14 y        | 7 y                         | 42                  | 0.46  | 9                 | c.1232A>Gp.(Asn411Ser) | Maternal  | CM1212157 | Pathogenic | Early onset absence seizures HP:0011152, hypoglycorrachia 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, hypertonia HP:0001252, microcephaly HP:0000252, myoclonus HP:0001336, hypoglycorrachia HP:0011972 |
| P7   | 27 y        | No data                     | 40                  | 0.49  | No data           | c.1202C>Gp.(Pro401Arg) | Not done  | New  | Likely pathogenic | Global developmental delay HP:0001263, seizure HP:0001250, dystonia HP:0001332, hypoglycorrachia 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:000543, global developmental delay HP:0001263, hypoglycorrachia 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, hypoglycorrachia 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, hypoglycorrachia HP:0011972 |
| P11  | 27 y        | 13 y                        | 32                  | 0.38  | 7                 | c.524G>Tp.(Gly175Val) | Not done  | New  | Likely pathogenic | Seizure HP:0001250, cognitive impairment HP:000543, scoliosis HP:0002650, hypoglycorrachia HP:0011972, abnormal cerebellum morphology HP:0001317 |
| REF. | Current age | Age at biochemical diagnostic* | CSF glucose (mg/dl) | Ratioa | CSF lactate (mg/dl) | Variants | Inheritance | HGMD | ACMGc | 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:0007750, abnormality of eye movement HP:0000496, oculogyric crisis HP:0010553, ataxia HP:0001251, abnormality of extrapyramidal motor function HP:0002071, short attention span HP:000736 |
|      |             |                                 |                     |         |                   |           |             |       |       |                                             |
| P13  | 24 y        | 13 y                           | 34                  | 0.39    | 10                | c.18+2T>G p.?    | De novo    | CS1411096 Pathogenic |                                             |
|      |             |                                 |                     |         |                   |           |             |       |       |                                             |
| 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:00007750, seizure HP:0001250, atypical absence seizure HP:0007270, dystonia HP:0001332, dystarthis 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:00007750, 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)
| REF. | Current age | Age at biochemical diagnostic | CSF glucose (mg/dl) | Ratio | CSF lactate (mg/dl) | Variants | Inheritance | HGMD | ACMG | Clinical data human phenotype ontology (HPO) |
|------|-------------|-------------------------------|----------------------|-------|---------------------|----------|-------------|-------|-------|---------------------------------------------|
| P21  | 6 y         | 4 m                           | 42                   | 0.38  | 8.7                 | c.1387A>C/p.(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:0000543, 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 |
| P25d | 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 |
| P26d | 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, impaired executive functioning HP:0033051, abnormal social behaviour HP:0012433 |
| 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)
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.\(^1\) 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.\(^16\)

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## TABLE 1

| Age at | Current | Age at | Biochemical | CSF | Lactate | Ratio | Variants |
|---|---|---|---|---|---|---|---|
| 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, bilateral tonic-clonic seizures |
| | | | | | | HP:0002694, generalized myoclonic sign |
| | | | | | | HP:0007256, tetraparesis |

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.

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Patient 25 and patient 26 are siblings.

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or to variants in SLC2A1 not detectable by the technology employed.
| REF. | Current age | Age at biochemical diagnostic | CSF glucose (mg/dl) | Ratio | CSF lactate (mg/dl) | Gene | Variants | Inheritance pattern | pLI | O/E | Inheritance | HGMD | ACMG | 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:007359, 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, |
| REF. | Current age | Age at biochemical diagnostic | CSF glucose (mg/dl) | CSF lactate (mg/dl) | Gene | Variants | Inheritance pattern | O/E | Inheritance | HGMD | ACMG | Clinical data human phenotype ontology (HPO) |
|------|-------------|-----------------------------|---------------------|-------------------|------|----------|---------------------|-----|-------------|-------|------|---------------------------------------------|
| P34  | 17 y        | 8 y                         | 46                  | 0.48              | 10.0 | P34      | SLC9A6 (NM_006359.3) |     | X-LR        |       |      | Severe global developmental delay |
|      |             |                             |                     |                   |      | c.803+1G>A | p.(Val233Alafs*3)  |     | Maternal    |       |      | HP:000711, impulsivity |
|      |             |                             |                     |                   |      |           |                     |     | CS1918586   |       |      | HP:0100710, impaired social reciprocity |
|      |             |                             |                     |                   |      |           |                     |     |                         |       |      | HP:0012760, EEG abnormality |
|      |             |                             |                     |                   |      |           |                     |     |                         |       |      | HP:0002353, hypoglycorrhachia |
|      |             |                             |                     |                   |      |           |                     |     |                         |       |      | HP:0011972 |
| P35  | 17 y        | 7 y                         | 46                  | 0.49              | 10.0 | P35      | NKX2-1 (NM_001079668.3) |     | AD          |       |      | Tremor HP:0001397, postural tremor |
|      |             |                             |                     |                   |      | c.727del | p.(Arg243Alafs*4) |     |             |       |      | HP:0002174, abnormal brain positron emission tomography |
|      |             |                             |                     |                   |      |           |                     |     |             |       |      | HP:0012657, specific learning disability |
|      |             |                             |                     |                   |      |           |                     |     |             |       |      | HP:0001328, dysgraphia |
|      |             |                             |                     |                   |      |           |                     |     |             |       |      | HP:0010526, Short attention span |

(Continues)
| REF. age | Age at biochem. diagnostic | CSF glucose (mg/dl) | Ratio | Gene | Variants | Inheritance | O/E | Inheritance | HGMD | ACMG | Clinical data human phenotype ontology (HPO) |
|---------|---------------------------|---------------------|-------|------|----------|-------------|-----|-------------|------|------|------------------------------------------|
| P36 6 y 1 y | 47 | 0.49 | 15.3 | ATP1A3 | c.2401G>A p.(Asp801Asn) | AD | 1 | 0 | De novo | CM127591 | Pathogenic | HP:0000736, hypothyroidism HP:0000821, poor fine motor coordination HP:0007010, chorea HP:0002072, dystonia HP:0001332, hypoglycorrhachia HP:0011972 |
| 46 | 0.55 | 13.7 | ATP1A3 | c.2401G>A p.(Asp801Asn) | AD | 1 | 0 | De novo | CM127591 | Pathogenic | HP:0000736, hypothyroidism HP:0000821, poor fine motor coordination HP:0007010, chorea HP:0002072, dystonia HP:0001332, hypoglycorrhachia HP:0011972 |
| P37 10 y 3 m | 40 | 0.46 | 7.9 | KCNQ2 | 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 |
| 45 | 0.47 | 12.7 | KCNQ2 | 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 |
| REF. | Age at biochemical diagnostic | Age at CSF | Ratio | CSF glucose (mg/dl) | Ratio | Gene | Variants | Inheritance pattern | pLI | O/E | Inheritance | HGMD | ACMG | Clinical data human phenotype ontology (HPO) |
|------|-------------------------------|------------|-------|---------------------|-------|------|----------|---------------------|-----|-----|-------------|-------|-------|-------------------------------------------|
| P38  | 11 y                          | No data    | 46    | 0.60                | 10.0  | SLC6A1| c.278_279del p.(Ala93Glyfs*113) | AD   | 1   | 0.03 | De novo | CD1918588 | Pathogenic |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | hypoglycorrhachia                          |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:00011972                               |
| P39  | 15 y                          | No data    | 46    | 0.56                | No data | NALCN| c.965T>C p.(Ile322Thr) | AD   | 0   | 0.4  | De novo | CM1611146 | Pathogenic |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | EEG abnormality                           |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:0002131, dystonia                       |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:00001332, hypotonia                     |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:00001252, paroxysmal dyskinesia         |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:0007166, hypermetropia                  |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:0000540, astigmatism                    |
|      |                               |            |       |                     |       |      |           |                     |      |     |              |       |       | HP:000483,                                     |
| REF. | Age | 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 |
| P40  | 13 y| 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 |
|      | 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 | |

| P41  | 26 y| Encephalopathy HP:0001298, intellectual disability HP:0001249, abnormality of coordination HP:0011443, involuntary movements HP:0004305, paroxysmal dyskinesia HP:0007166 |
|      | 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 | |
| REF. age | Current age | Age at biochemical diagnostic | CSF glucose (mg/dl) | Ratio | CSF lactate (mg/dl) | Gene | Gene Variants | Inheritance pattern | pLI | O/E | Inheritance | HGMD | ACMG | Clinical data human phenotype ontology (HPO) |
|----------|-------------|-------------------------------|--------------------|-------|-------------------|------|----------------|---------------------|------|-----|-------------|------|------|---------------------------------|
| P42      | 7 y         | 1 y                           | 20                 | 0.36  | 12.6              | MAN2B2 | c.2912C>T/ c.2912C>T | 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) |
| REF. | Current age | Age at biochemical diagnostic<sup>a</sup> | CSF glucose<sup>b</sup> (mg/dl) | Ratio<sup>b</sup> | Gene | Variants | Inheritance pattern | pLI | O/E | Inheritance | HGMD | ACMG<sup>c</sup> | Clinical data human phenotype ontology (HPO) |
|------|-------------|------------------------------------------|-------------------------------|----------------|------|----------|-------------------|-----|-----|-------------|-------|-----------|------------------------------------------------|
| P43  | 29 y        | No data                                 | No data                       | No data | NEXMIF| c.1882C>T | X-LD               | Not done | CM140386 | Pathogenic | Seizure HP:0001250, generalized-onset seizure |
| P44  | 9 y         | 2 y                                     | 44                            | 0.5      | 11.5 | UNC13A   | c.2422G>A | AD     | 1     | 0.09       | New | Uncertain significance | Seizure HP:0001250, Febrile seizure (within the age range of 3 months to 6 years) |

<sup>a</sup>Clinical data human phenotype ontology (HPO): HP:0003307, abnormal reflex; HP:0031826, hypoglycorrhachia; HP:0011972, 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:0002373, Global developmental delay; HP:0001263, Head tremor HP:0002346, Limb tremor HP:0200085, Action tremor HP:0002345, Stereotypical body rocking; HP:0012172, 52 S/C19 ANCHEZ-LIJARCIO ET AL.
| REF. age | Current age | Biochemical diagnostic | CSF glucose (mg/dl) | Ratio | CSF lactate (mg/dl) | Gene | Variants | Inheritance pattern | pLI | O/E | Inheritance | HGMD | ACMG | 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.

aAge at which the first lumbar puncture was performed.
bRatio: cerebrospinal fluid to serum blood glucose.

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/).
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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DATA AVAILABILITY STATEMENT
The data that support the findings reported here are available from the corresponding authors upon reasonable request.

REFERENCES
1. De Giorgis V, Veggiotti P. GLUT1 deficiency syndrome 2013: current state of the art. Seizure. 2013;22:803-811.
2. Klepper J, Scheffer H, Elsait MF, et al. Autosomal recessive inheritance of GLUT1 deficiency syndrome. Neuropediatrics. 2009;40:207-210.
3. De Vivo DC, Trifiletti RR, Jacobson RI, et al. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and development delay. N Engl J Med. 1991;325:703-709.
4. Klepper J, Akman C, Armeno M, et al. Glut1 deficiency syndrome (Glut1DS): state of the art in 2020 and recommendations of the international Glut1DS study group. Epilepsia Open. 2020;5:354-365.
5. Klepper J. Glucose transporter deficiency syndrome (GLUT1DS) and the ketogenic diet. Epilepsia. 2008;49(suppl 8):46-49.
6. Kossoff EH, Hartman AL. Ketogenic diets: new advances for metabolism-based therapies. *Curr Opin Neurol*. 2012;25:173-178.

7. Leen WG, Klepper J, Verbeek MM, et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain*. 2010;133:655-670.

8. Leen WG, Wevers RA, Kamsteeg E-J, et al. Cerebrospinal fluid analysis in the workup of GLUT1 deficiency syndrome: a systematic review. *JAMA Neurol*. 2013;70:1440-1444.

9. Köhler S, Carmody L, Vasilevsky N, et al. Expansion of the human phenotype ontology (HPO) knowledge base and resources. *Nucleic Acids Res*. 2019;47:D1018-D1027.

10. Klepper J. GLUT1 deficiency syndrome in clinical practice. *Epilepsy Res*. 2012;100:272-277.

11. Mayorga L, Gamboni B, Mampel A, et al. A frame-shift deletion in the PURA gene associates with a new clinical finding: hypoglycorrhachia. Is GLUT1 a new PURA target? *Mol Genet Metab*. 2018;123:331-336.

12. Eyster CA, Higginson JD, Huebner R, et al. Discovery of new cargo proteins that enter cells through Clathrin-independent endocytosis. *Traffic*. 2009;10:590-599.

13. Kremer LS, Wortmann SB, Proksch H. “Transcriptomics”: molecular diagnosis of inborn errors of metabolism via RNA-sequencing. *J Inherit Metab Dis*. 2018;41:525-532.

14. de la Morena-Barrio B, Stephens J, de la Morena-Barrio ME, et al. Long-read sequencing resolves structural variants in SERPINC1 causing antithrombin deficiency and identifies a complex rearrangement and a retrotransposon insertion not characterized by routine diagnostic methods. bioRxiv. 2020;271932. doi:10.1101/2020.08.28.271932

15. Fréard L, Montgomery SB. Diagnosing rare diseases after the exome. *Cold Spring Harb Mol Case Study*. 2018;4:a003392.

16. Lewis-Smith D, Galer PD, Balagura G, et al. Modeling seizures in the human phenotype ontology according to contemporary ILAE concepts makes big phenotypic data tractable. *Epilepsia*. 2021;62:1293-1305.

17. Harris JJ, Jolivet R, Attwell D. Synaptic energy use and supply. *Neuron*. 2012;75:762-777.

**SUPPORTING INFORMATION**

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