doi:10.1093/brain/awp336 Brain 2010: 133; 655–670 | 655

BRAIN
A JOURNAL OF NEUROLOGY

Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder

Wilhelmina G. Leen,[1] Joerg Klepper,[2] Marcel M. Verbeek,[1,3] Maike Leferink,[4] Tom Hofste,[4] Baziel G. van Engelen,[1] Ron A. Wevers,[3] Todd Arthur,[5] Nadia Bahi-Buisson,[6] Diana Ballhausen,[7] Jolita Bekhof,[8] Patrick van Bogaert,[9] Inês Carrilho,[10] Brigitte Chabrol,[11] Michael P. Champion,[12] James Coldwell,[13] Peter Clayton,[14] Elizabeth Donner,[15] Athanasios Evangeliou,[16] Friedrich Ebinger,[17] Kevin Farrell,[18] Rob J. Forsyth,[19] Christian G. E. L. de Goede,[20] Stephanie Gross,[21] Stephanie Grunewald,[22] Hans Holthausen,[23] Sandeep Jayawant,[24] Katherine Lachlan,[25] Vincent Laugel,[26] Kathy Leppig,[27] Ming J. Lim,[12] Grazia Mancini,[28] Adela Della Marina,[29] Loreto Martorell,[30] Joe McMenamin,[31] Marije E. C. Meuwissen,[28] Helen Mundy,[12] Nils O. Nilsson,[32] Axel Panzer,[33] Bwee T. Poll-The,[34] Christian Rauscher,[35] Christophe M. R. Rouselle,[36] Inger Sandvig,[37] Thomas Scheffner,[38] Eamonn Sheridan,[39] Neil Simpson,[40] Parol Sykora,[41] Richard Tomlinson,[42] John Trounce,[43] David Webb,[31] Bernhard Weschke,[44] Hans Scheffer[4,*] and Michél A. Willemsen[45,*]

1 Department of Neurology Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, The Netherlands
2 Department of Paediatrics and Paediatric Neurology, Children's Hospital, Aschaffenburg, Germany
3 Laboratory of Paediatrics and Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, The Netherlands
4 Department of Human Genetics, Radboud University Nijmegen Medical Centre, The Netherlands
5 Department of Child Neurology, Cincinnati Children's Hospital Medical Centre, Cincinnati, USA
6 Paediatric Neurology Necker Enfants Malades, APHP, Université Paris Descartes, Paris, France
7 Division of Molecular Paediatrics, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland
8 Department of Paediatrics, Isala Klinieken, Zwolle, The Netherlands
9 Department of Paediatric Neurology, ULB-Hôpital Erasme, Brussels, Belgium
10 Department of Paediatric Neurology, Children's Hospital Maria Pia, Porto, Portugal
11 Department of Neuropaediatrics Hôpital d'Enfants, CHU Timone, Marseille, France
12 Evelina Children's Hospital, London, UK
13 Children's Medical Centre, Tulsa, USA
14 UCL Institute of Child Health, London, UK
15 Division of Neurology, The Hospital for Sick Children, Toronto, Ontario, Canada
16 Department of Paediatrics, Papageorgiou Hospital, Thessaloniki, Greece
17 University Children's Hospital, Heidelberg, Germany
18 Department of Paediatrics, University of British Colombia and British Colombia's Children's Hospital, Vancouver, Canada
19 Institute of Neuroscience, Newcastle University, Newcastle, UK
20 Department of Paediatric Neurology, Royal Preston Hospital, Preston, UK
21 Department of Neuropaediatrics, Justus-Liebig-University, Giessen, Germany
22 Metabolic Department, Great Ormond Street Hospital for Children, London, UK
23 Neuropediatric Clinic, BHZ, Vogtareuth, Germany
24 Department of Paediatric Neurology, Oxford Children's Hospital, Oxford, UK
25 Department of Clinical Genetics, Wessex Regional Genetics Service, Southhampton, UK
26 Department of Paediatric Neurology, Strasbourg University Hospital, Strasbourg, France
27 Department of Genetics, Group Health Cooperative, Seattle, USA

Received September 14, 2009. Revised November 5, 2009. Accepted December 1, 2009. Advance Access publication February 2, 2010

28 Department of Clinical Genetics, Erasmus MC Rotterdam, The Netherlands
29 Department of Paediatric Neurology, University of Essen, Essen, Germany
30 Department of Molecular Genetics, Hospital Sant Joan de Déu, Barcelona, Spain
31 Department of Neurology, Our Lady's Children's Hospital, Crumlin, Dublin
32 Department of Paediatrics, Halmstad Hospital, Halmstad, Sweden
33 Department of Paediatric Neurology, DRK Kliniken, Berlin, Germany
34 Department of Paediatric Neurology, University Medical Centre, Amsterdam, The Netherlands
35 Department of Paediatrics, Paracelsus Medical University, Salzburg, Austria
36 Department of Paediatric Neurology, Hôpital Femme Mère Enfant, Lyon, France
37 Department of Paediatrics, University Hospital, Rikshospitalet, Oslo, Norway
38 Klinik für Kinder-und Jugend Medizin, Reutlingen, Deutschland
39 Leeds Institute of Molecular Medicine, Leeds, UK
40 Child Health Department, Bath NHS House, Bath, UK
41 Department of Child Neurology, University Hospital, Bratislava, Slovakia
42 Royal Devon and Exeter Hospital, Exeter, UK
43 Royal Alexandra Children's Hospital, Brighton, UK
44 Department of Neuropaediatrics, Charité-Universitätsmedizin Berlin, Berlin, Germany
45 Department of Paediatric Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, The Netherlands

*These authors contributed equally to this work.

Correspondence to: W. G. Leen, MD,
Department of Neurology
Radboud University Nijmegen Medical Centre
935 Neurology, PO BOX 9101
6500 HB Nijmegen
The Netherlands
E-mail: w.leen@neuro.umcn.nl

Glucose transporter-1 deficiency syndrome is caused by mutations in the *SLC2A1* gene in the majority of patients and results in impaired glucose transport into the brain. From 2004–2008, 132 requests for mutational analysis of the *SLC2A1* gene were studied by automated Sanger sequencing and multiplex ligation-dependent probe amplification. Mutations in the *SLC2A1* gene were detected in 54 patients (41%) and subsequently in three clinically affected family members. In these 57 patients we identified 49 different mutations, including six multiple exon deletions, six known mutations and 37 novel mutations (13 missense, five nonsense, 13 frame shift, four splice site and two translation initiation mutations). Clinical data were retrospectively collected from referring physicians by means of a questionnaire. Three different phenotypes were recognized: (i) the classical phenotype (84%), subdivided into early-onset (<2 years) (65%) and late-onset (18%); (ii) a non-classical phenotype, with mental retardation and movement disorder, without epilepsy (15%); and (iii) one adult case of glucose transporter-1 deficiency syndrome with minimal symptoms. Recognizing glucose transporter-1 deficiency syndrome is important, since a ketogenic diet was effective in most of the patients with epilepsy (86%) and also reduced movement disorders in 48% of the patients with a classical phenotype and 71% of the patients with a non-classical phenotype. The average delay in diagnosing classical glucose transporter-1 deficiency syndrome was 6.6 years (range 1 month–16 years). Cerebrospinal fluid glucose was below 2.5 mmol/l (range 0.9–2.4 mmol/l) in all patients and cerebrospinal fluid : blood glucose ratio was below 0.50 in all but one patient (range 0.19–0.52). Cerebrospinal fluid lactate was low to normal in all patients. Our relatively large series of 57 patients with glucose transporter-1 deficiency syndrome allowed us to identify correlations between genotype, phenotype and biochemical data. Type of mutation was related to the severity of mental retardation and the presence of complex movement disorders. Cerebrospinal fluid : blood glucose ratio was related to type of mutation and phenotype. In conclusion, a substantial number of the patients with glucose transporter-1 deficiency syndrome do not have epilepsy. Our study demonstrates that a lumbar puncture provides the diagnostic clue to glucose transporter-1 deficiency syndrome and can thereby dramatically reduce diagnostic delay to allow early start of the ketogenic diet.

Keywords: GLUT1 deficiency syndrome; *SLC2A1* gene; phenotype; cerebrospinal fluid; ketogenic diet

Abbreviations: GLUT1 = glucose transporter-1

Introduction

Glucose transporter-1 (GLUT1) deficiency syndrome (OMIM #606777) is an autosomal dominant haplo-insufficiency disorder, leading to a reduced glucose transport into the brain (Seidner *et al.*, 1998). GLUT1 is highly expressed in the endothelial cells of erythrocytes and the blood-brain barrier and is exclusively responsible for glucose transport into the brain (Vannucci *et al.*, 1997; Barros *et al.*, 2007). GLUT1 deficiency syndrome was first described in 1991 by De Vivo *et al*. (1991). The classic patient with GLUT1 deficiency syndrome presents with infantile drug-resistant seizures, mild to severe developmental delay and an acquired microcephaly in up to 50% of the cases. Hypotonia, spasticity, ataxia and dystonia are elements of a complex movement disorder (Klepper and Leiendecker, 2007). In recent years, however, is has become apparent that the clinical spectrum of GLUT1 deficiency syndrome is much broader (Brockmann, 2009). Patients with a non-classical phenotype featuring developmental delay and movement disorders without epilepsy have been described (Overweg-Plandsoen *et al.*, 2003; Hennecke *et al.*, 2005; Friedman *et al.*, 2006; Klepper *et al.*, 2007), as well as familial and sporadic paroxysmal exercise induced dyskinesia with or without epilepsy (Suls *et al.*, 2008; Weber *et al.*, 2008; Schneider *et al.*, 2009).

When GLUT1 deficiency syndrome is suspected on clinical grounds, a lumbar puncture in the fasting state should be performed. GLUT1 deficiency syndrome is characterized by a low glucose concentration in the cerebrospinal fluid (hypoglycorrhachia) in the absence of hypoglycaemia, in combination with a low to normal lactate in the cerebrospinal fluid (Klepper *et al.*, 1999*b*; Klepper and Voit, 2002). It is important to recognize GLUT1 deficiency syndrome, since the disorder can be treated with a ketogenic diet. Ketone bodies use another transporter to enter the central nervous system and thus can supply the brain with an alternative fuel.

In most patients the ketogenic diet markedly reduces the frequency of seizures (Klepper *et al.*, 2005) and the severity of the movement disorder (Brockmann, 2009). Behaviour and alertness also frequently improve (Klepper *et al.*, 2005, 2007; Joshi *et al.*, 2008).

For the diagnosis of GLUT1 deficiency syndrome, GLUT1 western blot analysis in erythrocyte membranes and glucose uptake into erythrocytes can be applied to determine GLUT1 function. Negative western blots or erythrocyte uptake studies, however, do not rule out the diagnosis (Klepper *et al.*, 1999*a*). GLUT1 deficiency syndrome can be confirmed by mutation analysis of the *SLC2A1* gene. The *SLC2A1* gene, mapped to the short arm of chromosome 1 (1p35-31.3) (Shows *et al.*, 1987), is a relatively small gene and consists of ten exons, spanning 2842 base pairs encoding 493 amino acids (Fukumoto *et al.*, 1988).

The structure and function of the GLUT1 protein has been intensively investigated (Hruz and Mueckler, 2001). The amino-acid sequence of the GLUT1 protein is highly conserved, with 97–98% identity between the human, rat, rabbit, mouse and pig sequences, which implies that all domains of this protein are functionally important (Baldwin, 1993). GLUT1 has 12 transmembrane alpha-helices (Mueckler *et al.*, 1985), separated by a large intracellular loop between helices 6 and 7. A 3D model for GLUT1 has been proposed, predicting a central aqueous channel communicating the extracellular and intracellular compartments, with many residues crucial for glucose transport located around this central channel (Mueckler and Makepeace, 2009).

Since the first description of GLUT1 deficiency syndrome and the elucidation of its genetic cause, a wide spectrum of around 60 different mutations in the *SLC2A1* gene has been described in approximately 100 patients (Wang *et al.*, 2005; Klepper and Leiendecker, 2007), including large-scale deletions, missense, nonsense, frame shift and splice-site mutations. All mutations either lead to absence or loss of function of one of the *SLC2A1* alleles. Several hot spots for recurrent mutations have been identified (Asn34, Gly91, Ser113, Arg126, Arg153, Arg264, Thr295, Arg333) (Klepper and Leiendecker, 2007). Thus far, correlations between phenotype and genotype remained elusive (Wang *et al.*, 2005; Brockmann, 2009). Here we report on the genetic, biochemical and clinical characteristics of 57 newly diagnosed patients with GLUT1 deficiency syndrome.

Materials and methods

Patients

From January 2004 to July 2008 the DNA Diagnostics Laboratory (Department of Human Genetics of the Radboud University Nijmegen Medical Centre, The Netherlands) received 132 requests for mutational analysis of the *SLC2A1* gene in patients suspected to have GLUT1 deficiency syndrome from all over the world (excluding the requests for carrier testing in parents and other family members of affected patients). In 54 patients (41%) suspicion of GLUT1 deficiency syndrome was confirmed by the identification of a pathogenic mutation in the *SLC2A1* gene. Additionally, we identified a mutation in the *SLC2A1* gene in three clinically affected family members, bringing the total number of patients with GLUT1 deficiency syndrome confirmed by mutation analysis to 57. Clinical data were retrospectively collected from referring physicians by means of a written, detailed questionnaire. Results were reviewed against the background of the existing literature on *SLC2A1* gene mutations and associated phenotypes.

Mutation analysis

Genomic DNA was extracted from blood samples by standard procedures. Automated Sanger sequencing was performed to study the entire coding sequence including at least 20 base pairs of intronic sequence flanking each exon of the *SLC2A1* gene. Polymerase chain reaction (PCR) amplification of all 10 exons of the *SLC2A1* gene was performed using Fast PCR Master Mix (Applied Biosystems, Foster City, California, USA). Primer sequences are available upon request. Sequencing reactions were carried out using BigDye V. 3.1 (Applied Biosystems) and analysed on an ABI 3730 capillary sequencer (Applied Biosystems). Sequences were compared to the wild-type sequence as submitted to Genbank (Genbank Accession Number NM_006516). All previously unreported mutations were verified in a panel of at least 100 control alleles. The reference sequence was used in which the A of the ATG start-codon is designated position 1. Amino-acid residues were numbered from the first methionine residue; according to the protein accession number NP_006507.

Single or multiple exon deletions or duplications of the *SLC2A1* gene using multiplex ligation-dependent probe amplification were analysed in all patients. Multiplex ligation-dependent probe amplification is a technique for measuring allele dosage and identifies the target sequence by hybridization of two adjacent complementary probes (Schouten *et al.*, 2002). Multiplex ligation-dependent probe amplification was carried out using the SALSA MLPA kit P138 *SLC2A1* (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions. A visual comparison of the peak profiles was made to identify copy number changes. Heterozygous deletions of probe recognition sequences should give 35–50% reduced relative peak area of the amplification product of that probe.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences version 16.0 (SPSS INC, Chicago, IL, USA). Mean differences in CSF parameters between patients with a different genotype or phenotype were assessed by means of analysis of variance (one-way ANOVA) using planned contrasts for group comparisons. Correlation between phenotype and genotype was tested using the Pearson Chi square test (two-sided testing for a significance level of $P \leqslant 0.05$).

Results

Molecular data

Out of 57 patients, 6 (11%) were identified with a multiple exon deletion of the *SLC2A1* gene detected by multiplex ligation-dependent probe amplification. In 51 patients a pathogenic mutation in the *SLC2A1* gene was identified by DNA sequence analysis (Table 1). We identified 37 different novel pathogenic mutations, including 13 missense, 5 nonsense, 13 frame shift, 4 splice and 2 translation initiation mutations. We identified two different *de novo* mutations in amino-acid residue 1 (c.1A>G and c.3G>A). Since the mutations were located in the transcription codon of the *SLC2A1* gene, transcription was not initiated, which is consistent with haplo-insufficiency. We identified 17 different missense mutations in the *SLC2A1* gene, 13 of which were novel. Eleven novel missense mutations were identified in patients with the classical phenotype of GLUT1 deficiency syndrome of epilepsy and mental retardation in combination with movement disorders (p.Asn34Tyr, p.Met96Val, p.Ala155Val, p.Arg212Cis, p.Arg212His, p.Arg223Trp, p.Glu329Gln, p.Arg333Gln, p.Gly382Asp, p.Ala405Asp, p.Pro485Leu). The novel missense mutation p.Arg153His was identified in Patient 48 with the non-classical phenotype of mild mental retardation with continuous ataxia and paroxysmal dystonia and choreoathetosis. CSF in this patient showed a characteristic pattern of low CSF glucose (1.8 mmol/l) in combination with low CSF : blood glucose ratio (0.38) and low CSF lactate (1.0 mmol/l). The novel missense mutation p.Val303Leu was identified in Patient 57. This patient was lost as follow-up and clinical data were unavailable. DNA of the parents of this patient was not investigated.

All previously unreported mutations were verified in a panel of at least 100 control alleles. Mutations were not mentioned as non-pathogenic polymorphism in the Single Nucleotide Polymorphism database. Two mutation prediction programs were used to predict the pathogenicity of mutations [PolyPhen (Polymorphism Phenotyping) and SIFT (Sorting Intolerant from Tolerant)]. The mutations that we identified and all mutations in the *SLC2A1* gene that have been described previously are represented in Fig. 1 (Klepper *et al.*, 2007; Klepper and Leiendecker, 2007; Ito *et al.*, 2008; Joshi *et al.*, 2008; Roulet-Perez *et al.*, 2008; Suls *et al.*, 2008; Takahashi *et al.*, 2008; Ticus *et al.*, 2008; Weber *et al.*, 2008; Zorzi *et al.*, 2008; Bertsche *et al.*, 2009; Slaughter *et al.*, 2009).

Autosomal dominant transmission

We identified four families with autosomal dominant transmission of GLUT1 deficiency syndrome. Patient 11 (Table 2), the mother of Patient 49 (Table 4) was diagnosed with GLUT1 deficiency syndrome after diagnosis was made in her daughter. Whereas her daughter had a non-classical phenotype, with mild mental retardation with continuous ataxia with paroxysmal worsening, the mother had the early-onset classical phenotype of epilepsy with mild mental retardation and paroxysmal ataxia.

Patient 55, the mother of Patient 23 (Table 2), was diagnosed with GLUT1 deficiency syndrome after diagnosis was made in her son. The son had the early-onset classical phenotype with a mild mental retardation without movement disorders, whereas the mother had a minimal phenotype with a normal psychomotor development and 'clumsiness' after prolonged fasting.

The missense mutation Arg93Trp was also identified in the brother and mother of Patient 50 (Table 4). Patient 50 had a non-classical phenotype of severe mental retardation with chorea. The brother had epilepsy and a mild mental retardation. The mother had epilepsy and a normal intelligence within the lower range (based on the clinical impression of the treating physician).

In one other family the mutation in the *SLC2A1* gene was most likely transmitted in an autosomal dominant way. A mutation in the *SLC2A1* gene was identified in Patient 45 (Table 3) after diagnosis of GLUT1 deficiency syndrome was made in his brother (Patient 15, Table 2). Patient 15 had the early-onset classical phenotype with severe mental retardation, whereas Patient 45 had the late-onset classical phenotype with only mild mental retardation. DNA of their mother was investigated and did not show the mutation. Clinical data and DNA of the father were unavailable.

In all other cases where both parents had been tested ($n = 11$; 22%) the mutation had occurred as a *de novo* event.

Biochemical characteristics

The biochemical characteristics of the patients are listed in Tables 2—4. All patients had low CSF glucose concentrations (<2.5 mmol/l). The CSF glucose values (reference range 2.5–3.7 mmol/l) were 0.9–2.4 mmol/l (mean 1.8 mmol/l; SD 0.3). CSF : blood glucose ratios (reference range 0.5–0.8) ranged from 0.19 to 0.52 (mean 0.37; SD 0.07). CSF lactate (reference range 1.3–1.9 mmol/l) ranged from 0.6 to 1.5 mmol/l (mean 1.0 mmol/l; SD 0.2), with a CSF lactate below 1.3 mmol/l in 78% of the patients.

Table 1 Mutations in the *SLC2A1* gene in 51 novel GLUT1 deficiency syndrome patients

Exon	Nucleotide	Amino acid	Location	Type of mutation	Patient number	Phenotype	References[a]
1	c.1A>G	p.Met1?	Transcription codon	B	42	B	–
1	c.3G>A	p.Met1?	Transcription codon	B	26	A	–
Intron 1	c.18+1G>C	–	–	B	24	A	–
Intron 1	c.18+1G>A	–	–	B	23, 55	A, minimal	Wang *et al*., 2000
2	c.32_33del	–	Helix 1	B	21	A	–
2	c.100A>T	p.Asn34Tyr	Extracellular loop 1–2	A	1	A	–
Intron 2	c.115-1G>A	–	–	B	17	A	–
3	c.124G>T	p.Glu42X	Extracellular loop 1–2	B	46	B	–
3	c.160G>T	p.Glu54X	Extracellular loop 1–2	B	54	C	–
3	c.170dup	–	Extracellular loop 1–2	B	18	A	–
4	c.277C>T	p.Arg93Trp	Cytoplasmic loop 2–3	A	50	C	Joshi *et al*., 2008
4	c.286A>G	p.Met96Val	Helix 3	A	3	A	–
4	c.305_308del	–	Helix 3	B	43	B	–
4	c.338_352delinsTTGAG	–	Helix 3	B	13	A	–
4	c.354_355insT	–	Extracellular loop 3–4	B	14	A	–
4	c.376C>T	p.Arg126Cys	Helix 4	A	6, 11, 47, 49	A, A, C, C	Pascual *et al*., 2002; Zorzi *et al*., 2008
4	c.388G>A	p.Gly130Ser	Helix 4	A	5	A	Wang *et al*., 2005
4	c.458G>A	p.Arg153His	Cytoplasmic loop 4–5	A	48	C	–
4	c.464C>T	p.Ala155Val	Helix 5	A	39, 37	B, B	–
4	c.481C>T	p.Gln161X	Helix 5	B	28	A	–
4	c.505_507del	–	Helix 5	B	20	A	Pascual *et al*.2002; Wang *et al*., 2005
5	c.634C>T	p.Arg212Cys	Cytoplasmic loop 6–7	A	8	A	–
5	c.635G>A	p.Arg212His	Cytoplasmic loop 6–7	A	7	A	–
5	c.667C>T	p.Arg223Trp	Cytoplasmic loop 6–7	A	40	B	–
6	c.715_716insC	–	Cytoplasmic loop 6–7	B	25	A	–
6	c.727G>T	p.Glu243X	Cytoplasmic loop 6–7	B	16	A	–
6	c.737_741del	–	Cytoplasmic loop 6–7	B	56	NA	–
6	c.746del; 746-747ins9	–	Cytoplasmic loop 6–7	B	15, 45	A, B	–
6	c.790dup	–	Cytoplasmic loop 6–7	B	19	A	–
6	c.798_799insC	–	Cytoplasmic loop 6–7	B	12	A	–
6	c.844C>T	p.Gln282X	Helix 7	B	29	A	–
Intron 6	c.864-1G>C	–	–	B	52	C	–
7	c.884C>T	p.Thr295Met	Extracellular loop 7–8	A	9	A	Wang *et al*., 2005; Fuji *et al*., 2008
7	c.907G>T	p.Val303Leu	Extracellular loop 7–8	A	57	NA	–
7	c.966_971delinsT	–	Helix 8	B	51	C	–
8	c.985G>C	p.Glu329Gln	Cytoplasmic loop 8–9	A	4	A	–
8	c.988C>T	p.Arg330X	Cytoplasmic loop 8–9	B	30, 44	A, B	Wang *et al*., 2000; Ito *et al*., 2008
8	c.998G>A	p.Arg333Gln	Cytoplasmic loop 8–9	A	38	B	–
9	c.1100dup	–	Extracellular loop 9–10	B	53	C	–
9	c.1145G>A	p.Gly382Asp	Helix 10	A	2	A	–
9	c.1214C>A	p.Ala405Asp	Helix 11	A	41	B	–
Intron 9	c.1279-1G>C	–	–	B	22	A	–
10	c.1346_1359del	–	Helix 12	B	27	A	–
10	c.1454C>T	p.Pro485Leu	Cytoplasmic tail	A	10	A	–

Type of mutation: A = missense mutation, B = nonsense, frame shift, splice site, or translation initiation mutation. Six patients with a multiple exon deletion of the *SLC2A1* gene (deletion of exon 2–10 in Patient 34 and deletion of exon 1–10 in Patients 31, 32, 33, 35 and 36) are not represented in the table. Phenotype: A = early-onset classical phenotype; B = late-onset classical phenotype; C = non-classical phenotype; NA = data not available.
a: Patients with the same mutation described previously in the literature.

Clinical characteristics

We obtained clinical data of 55 patients (96%). The clinical characteristics are listed in Tables 2–4. Two patients (56 and 57) were lost to follow-up and clinical data were unavailable. We recognized three different phenotypes. The most frequent, 'classical' phenotype ($n = 46$; 84%) represented patients with refractory epilepsy and developmental delay. Patients generally had an onset of

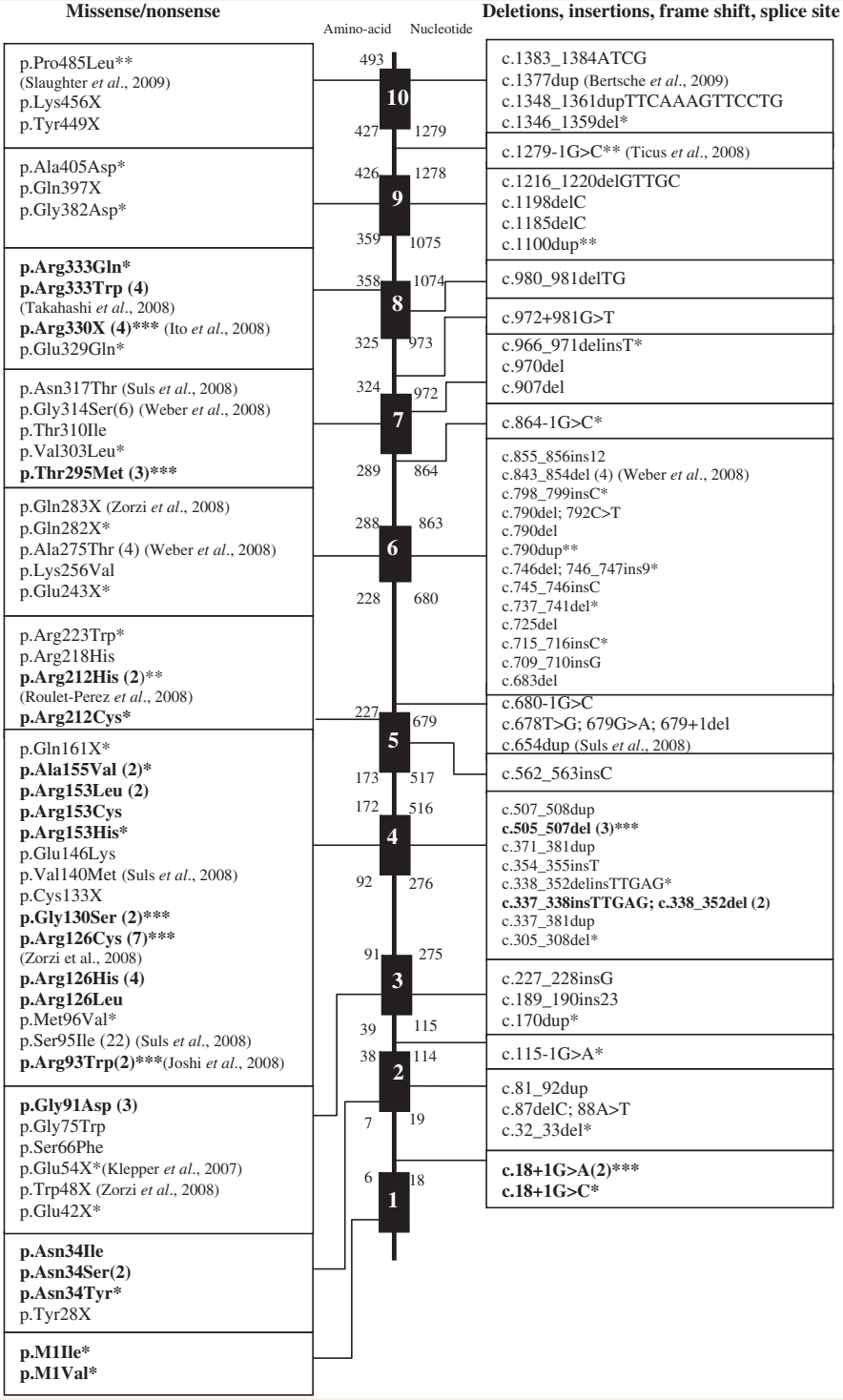

Figure 1 Distribution of 100 different pathogenic mutations in the *SLC2A1* gene in 162 patients with GLUT1 deficiency syndrome that were identified by us or have previously been described in literature. Six patients that we identified with a heterozygous multiple exon deletion and five patients that were described previously with haplo-insufficiency of the *SLC2A1* gene are not represented. The black vertical line represents the GLUT1 gene with boxes indicating the exons. The left hand diagram shows missense and nonsense mutations. Amino-acid residues at the intron/exon boundaries are shown. The right hand diagram shows frame shift and splice-site mutations. Nucleotides at the intron–exon boundaries are indicated. Genbank Accession Number NM_006516 was used as the SLC2A1 reference sequence in which the A of the ATG start-codon is designated position 1. Some mutations have been reported in the past with a different reference sequence (in which the A of the ATG start-codon is +180); we have adapted these mutations to the reference sequence as mentioned above. The number between brackets represents total number of patients identified with the mutation. Mutation hot spots are represented in bold. *Novel mutation identified in our patient group. **Novel mutation identified in one of our patients that has previously been reported as a case report. ***Mutation identified in our patient group that has previously been reported in other patients. References of mutations that have been described by others after 2007 are mentioned. All other mutations have been described previously by Klepper and Leiendecker (2007).

Table 2 Characteristics of 36 novel GLUT1 deficiency syndrome patients with the early-onset classical phenotype

Patient characteristics		Type of mutation	Biochemical data			Clinical data									Effect of ketogenic diet on		
Patient	Sex; age		CSF : blood glucose ratio	CSF blood glucose	CSF lactate	Age at diagnosis	Seizures; onset/ frequency	Microcephaly	Mental retardation	Hypotonia	Pyramidal signs	Movement disorder			Seizures	Movement disorder	Cognition
	years			mmol/l	mmol/l	years	months					Ataxia	Dystonia	Chorea			
1	F; 1.4	A	0.28	1.3	0.6	0.2	1/W	–	Mild	+	–	–	–	–	+	N/A	–
2	M; 4	A	0.30	1.7	1.4	0.4	4/M	+	Sev	+	+	–	P	–	+/–	–	–
3	F; 8	A	0.31	2.0	1.0	7	15/D	–	Mild	–	–	–	–	–	+/–	N/A	+
4	F; 3	A	<0.4	NA	NA	NA	6/NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	F; 19	A	0.40	2.0	0.9	16	4/W	+	Mild‡	–	–	P	P	–	+	+/–	–
6	M; 21	A	0.41	2.1	1.1	17	3/D	–	Mod	–	+	C	–	–	–	+/–	+
7[a]	F; 12	A	0.42	1.9	1.1	9	3/D	–	Mild	–	–	–	–	–	+	N/A	+
8	F; 19	A	0.43	2.1	1.2	17	15/D	NA	Mild‡	NA	–	–	–	–	NA	NA	NA
9	M; 12	A	0.43	2.4	1.3	9	3/M	–	Mild‡	–	+	C/P	P	–	–	+/–	+
10[a]	F; 11	A	0.45	2.0	NA	9	12/D	–	Mild	–	–	–	–	–	+/–	N/A	+
11	F; 34	A	NA	NA	NA	33	6/W	NA	Mild	–	–	P	–	–	+/–(MA)	–	–
12	M; 4	B	0.19	1.1	0.9	0.4	2/D	–	Mild	+	–	–	–	–	+	N/A	+
13	M; 7	B	0.25	1.3	0.6	4	3/D	–	Mod‡	–	+	P	C	C	+	–	–
14	F; 5	B	0.28	1.3	0.9	0.8	1/W	+	Mild	+	–	C	–	–	+	–	+
15	M; 16	B	0.29	1.8	1.1	14	23/M	+	Sev‡	+	–	C/P	–	–	0	0	0
16	F; 3	B	0.30	1.4	1.0	2	2/D	+	Mod‡	–	–	C	–	–	+	+/–	+
17	M; 17	B	0.31	1.3	NA	15	2/M	NA	Sev‡	–	–	C	–	–	0	0	0
18	M; 12	B	0.31	2.2	NA	11	13/D	+	Mod	+	+	–	–	–	0	0	0
19	F; 16	B	0.32	1.4	0.7	14	3/D	–	Mod	–	+	C	–	–	+/–	–	–
20	M; 7	B	0.36	1.6	NA	4	3/W	–	Mod	+	–	C	–	–	+	–	–
21	M; 7	B	0.36	1.7	1.2	4	9/W	–	Mod‡	+	–	C	C/P	–	+	+/–	+
22[a]	M; 5	B	0.37	1.9	NA	2	4/D	+	Mod‡	+	–	P	P	–	+	+/–	–
23	M; 5	B	0.38	1.7	NA	4	3/NA	–	Mild	–	–	–	–	–	+	N/A	–
24	F; 19	B	0.38	1.8	0.7	14	6/D	–	Sev	NA	+	C	–	–	+/–; S	–; S	–; S
25	M; 8	B	0.39	2.0	0.7	4	8/S	+	Mod	+	+	C	C	–	+	+/–	+
26	M; 12	B	0.38	1.8	1.0	7	11/NA	+	Mod	–	–	+	–	–	+/–	–	+
27	M; 24	B	0.40	2.1	1.5	20	4/D	–	Mild‡	+	–	P	–	–	+	+/–	–
28	M; 14	B	NA	NA	NA	NA	6/S	+	Mod	+	+	C	C	–	0	0	0
29	F; 10	B	NA	1.5	1.1	8	14/S	–	Mod‡	+	+	C	C	–	+/–(MA)	+/–	–
30	F; 13	B	NA	NA	NA	11	2/D	+	Sev	+	–	C	–	–	–	–	+
31	F; 6	C	0.20	0.9	NA	5	+	–	Mild	–	–	–	C	–	+	–	+
32	F; 2	C	0.29	1.3	0.9	0.7	3/D	–	Mod‡	+	–	–	–	–	+	N/A	+
33	F; 7	C	0.32	1.6	0.9	6	6/S	+	Mod	+	+	P	P	–	+; S	+; S	–; S
34	M; 3	C	0.35	1.9	1.5	1.7	2/W	+	Mod‡	+	+	–	C	–	+	+/–	+
35	F; 9	C	0.37	1.6	1.5	5	12/W	–	Sev	+	+	C	–	–	+	+/–	–

(continued)

Table 2 Continued

Patient characteristics		Type of mutation	Biochemical data			Clinical data									Effect of ketogenic diet on		
Patient	Sex; age		CSF : blood glucose ratio	CSF blood glucose	CSF lactate	Age at diagnosis	Seizures; onset/ frequency	Microcephaly	Mental retardation	Hypotonia	Pyramidal signs	Movement disorder			Seizures	Movement disorder	Cognition
	years			mmol/l	mmol/l	years	months					Ataxia	Dystonia	Chorea			
36	M; 10	C	0.39	1.6	NA	7	5/D	+	Mod	–	+	C/P	–	P	+	–	–
Total (mean; range)	M 50% F 50% 10.7; 1.4–34	A 31% B 53% C 17%	0.36; 0.19–0.45	1.7; 0.9–2.4	1.0; 0.6–1.5	8.3; 0.2–33	6.3; 1–23	+ 39% – 50% NA 11%	Mild 36% Mod 44% Sev 17% NA 3%	+ 50% – 42% NA 8%	+ 39% – 58% NA 3%	C 36% P 17% C/P 8% + 3% – 33% NA 3%	C 17% P 14% C/P 3% – 64% NA 3%	C 3% P 3% C/P 0% – 92% NA 3%	+ 53% +/– 22% – 8% 0 11% S 6% NA 6%	+ 3% +/– 31% – 31% 0 11% S 6% NA 6% N/A 19%	+ 42% – 42% 0 11% NA 6%

Three patients are not described in the table: Patient 55 had a minimal phenotype; clinical data from Patients 56 and 57 were unavailable.

Sex: M = male; F = female. Type of mutation: A = missense mutation; B = nonsense, frame shift, splice site, or translation initiation mutation; C = multiple exon deletion. Seizure frequency: D = daily; W = weekly; M = monthly; S = sporadic. Microcephaly: + = more than two standard deviations below the mean; – = within two standard deviations from the mean. Mental retardation: mild = IQ 50–70; mod = moderate (IQ 35–49); sev = severe (IQ 20–34); ‡ = IQ test was not performed, the severity of mental retardation was based on school level and/or clinical impression of the treating physician. Movement disorder: C = continue; P = paroxysmal; C/P = continue with paroxysmal worsening. Effect of ketogenic diet on (i) seizures: + = seizure free; +/– = reduction of seizures; – = no reduction of seizures; (ii) movement disorder: + = total disappearance of movement disorder; +/– = reduction of frequency and/or severity of movement disorder; – = no effect on movement disorder; (iii) cognition: + = subjective improvement of alertness and behaviour; – = no change in alertness or behaviour; S = ketogenic diet was stopped/patient was non-compliant; 0 = ketogenic diet was not started; MA = modified Atkins diet. NA = data not available; N/A = not applicable.

a: Four patients were previously published as a case report: 7 (Roulet-Perez *et al*., 2008); 10 (Slaughter *et al*., 2009); 22 (Ticus *et al*., 2008); 54 (Klepper *et al*., 2007).

Table 3 Characteristics of 10 novel GLUT1 deficiency syndrome patients with the late-onset classical phenotype

Patient characteristics		Type of mutation	Biochemical data			Clinical data									Effect of ketogenic diet on		
Patient	Sex; age		CSF: blood glucose ratio	CSF blood glucose	CSF lactate	Age at diagnosis	Seizures; onset/ frequency	Microcephaly	Mental retardation	Hypotonia	Pyramidal signs	Movement disorder					
	years			mmol/l	mmol/l	years	months					Ataxia	Dystonia	Chorea	Seizures	Movement disorder	Cognition
37	M; 14	A	0.35	1.8	1.2	13	72/D	–	Mild	–	–	P	–	P	S	S	S
38	F; 13	A	0.40	2.0	NA	12	60/D	NA	Mod‡	–	–	P	–	–	+	+	+
39	F; 7	A	0.43	2.3	1.3	4	30/D	NA	Mild‡	–	–	–	–	–	+	N/A	–
40	F; 5	A	0.46	2.1	1.3	3	24/D	+	Mild‡	–	–	C	–	–	+/–	–	–
41	F; 21	A	0.52	2.2	1.4	16	36/D	+	Mild	–	–	P	–	–	0	0	0
42	F; 17	B	0.3	1.9	1.1	15	44/D	–	Mild	+	+	C	P	–	+	+/–	+
43	M; 7	B	0.36	1.7	NA	4	27/S	–	Mod	+	–	C	–	–	+	–	+
44	F; 6	B	0.38	1.3	1.2	2	60/S	–	Mod‡	+	–	–	–	P	–	+/–	+
45	M; 12	B	0.38	1.8	0.9	10	108/M	–	Mild	–	–	P	–	–	0	0	0
46	F; 13	B	0.47	2.2	0.9	11	132/S	NA	Mild	+	–	C/P	–	–	0	0	0
Total (mean; range)	M 30% F 70% 11.5; 5–21	A50% B 50% C 0%	0.41; 0.3–0.52	1.9; 1.3–2.3	1.2; 0.9–1.4	9.0; 2–16	59; 24–132	+ 20% – 50% NA 30%	Mild 70% Mod 30% Sev 0%	+ 40% – 60%	+ 10% – 90%	C 30% P 40% C/P 10% – 20%	C 0% P 10% C/P 0% – 90%	C 0% P 20% C/P 0% – 80%	+ 40% +/– 10% – 10% 0 30% S 10%	+ 10% +/– 20% – 20% 0 30% S 10% N/A 10%	+ 40% –20% 0 30% S 10%

Three patients are not described in the table: Patient 55 had a minimal phenotype; clinical data of Patients 56 and 57 were unavailable. Abbreviations same as in Table 2 footnote.

Table 4 Characteristics of 8 novel GLUT1 deficiency syndrome patients with the non-classical phenotype

Patient characteristics		Type of mutation	Biochemical data			Clinical data									Effect of ketogenic diet on		
Patient	Sex; age		CSF: blood glucose ratio	CSF blood glucose	CSF lactate	Age at diagnosis	Seizures; onset/ frequency	Microcephaly	Mental retardation	Hypotonia	Pyramidal signs	Movement disorder					
	Years			mmol/l	mmol/l	years	months					Ataxia	Dystonia	Chorea	Seizures	Movement disorder	Cognition
47	M; 5	A	0.35	1.6	1.1	4	–	–	Mild	+	–	C	P	–	N/A	+/–	–
48	M; 7	A	0.38	1.8	1.0	6	–	–	Mild‡	–	+	C	P	P	N/A	S	S
49	F; 13	A	0.43	2.0	0.7	11	–	+	Mild‡	–	+	C/P	–	–	N/A	+/–	–
50[b]	M; 10	A	0.47	2.0	1.0	9	–	–	Sev	+	–	–	–	C	N/A	0	0
51	F; 7	B	0.37	1.8	0.9	4	–	–	Sev‡	–	+	+	–	+	N/A	+/–	–
52	M;10	B	0.38	1.8	NA	9	–	+	Mod	+	–	C	C	–	N/A	+/–	–
53	F; 3	B	0.40	1.5	NA	1.7	–	+	Mod	+	+	–	P	–	N/A	–	+
54[a]	M; 7	B	0.46	NA	NA	3	–	+	Mild	+	+	C	–	–	N/A	+/–	+
Total (mean; range)	M 63% F 38% 7.8; 3–13	A 50% B 50% C 0%	0.41; 0.35–0.47	1.8; 1.5–2.0	0.9; 0.7–1.1	6.0; 1.7–11	– 100%	+ 50% – 50%	Mild 50% Mod 25% Sev 25%	+ 63% – 38%	+ 63% – 38%	C 50% P 0% C/P 13% + 13% – 25%	C 13% P 38% C/P 0% – 50%	C 13% P 13% C/P 0% + 13% – 63%	N/A 100%	+ 0% +/– 63% – 13% 0 13%	+ 25% – 50% 0 13% S 13%

Three patients are not described in the table: Patient 55 had a minimal phenotype; clinical data from Patients 56 and 57 were unavailable. Abbreviations same as in Table 2 footnote.
a: Four patients were previously published as a case report: 7 (Roulet-Perez *et al.*, 2008); 10 (Slaughter *et al.*, 2009); 22 (Ticus *et al.*, 2008); 54 (Klepper *et al.*, 2007).
b: Patient also carried a mutation in the RYR2-gene (Phe2307Leu in exon 45) with the phenotype of catecholaminergic polymorphic ventricular tachycardia.

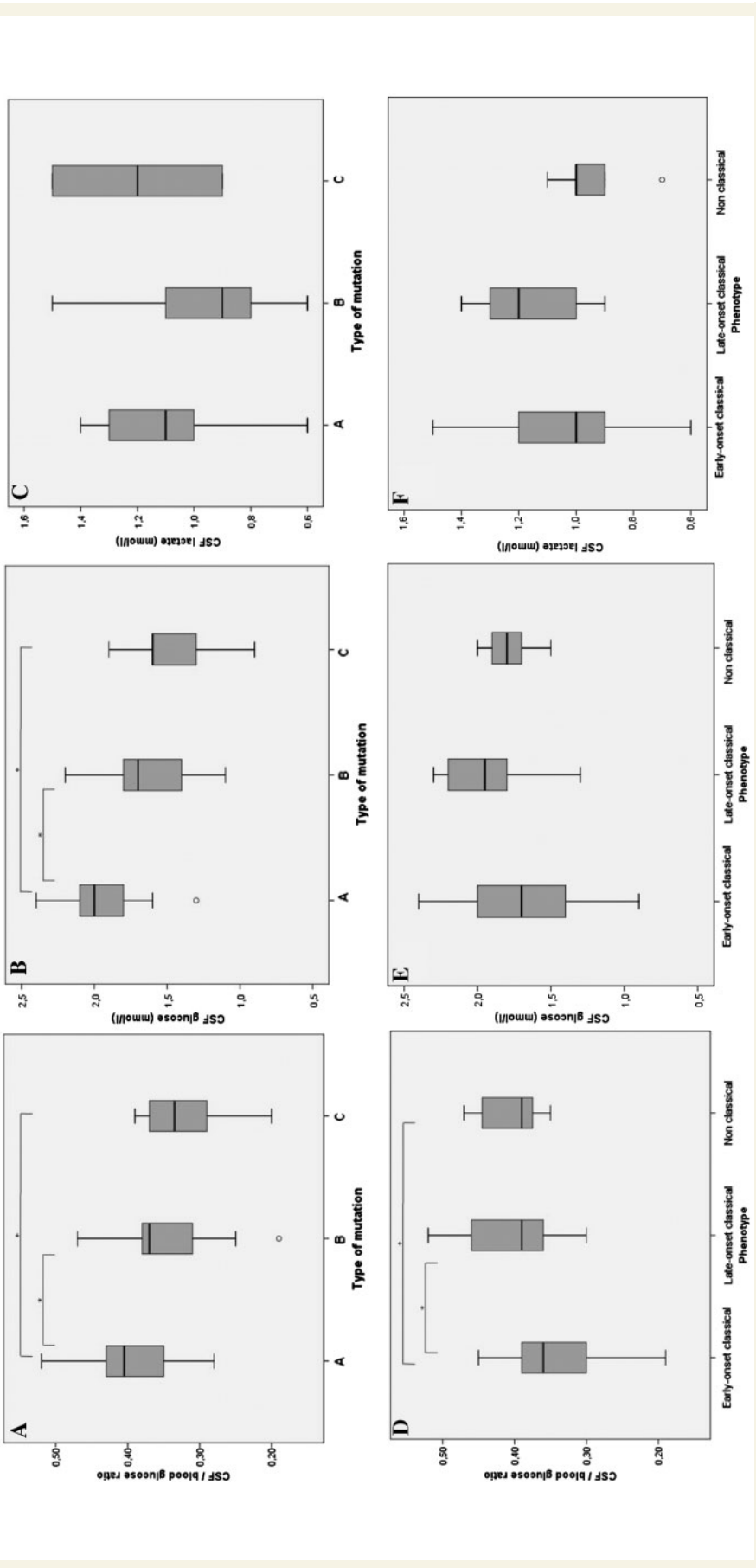

Figure 2 Correlation between type of mutation and CSF : blood glucose ratio (**A**); CSF glucose (**B**); and CSF lactate (**C**); and the correlation between phenotype and CSF : blood glucose ratio (**D**); CSF glucose (**E**); and CSF lactate (**F**). Reference range: CSF : blood glucose ratio 0.5–0.8; CSF glucose 2.5–3.7 mmol/l; CSF lactate 1.3–1.9 mmol/l. Type of mutation: A = missense mutation; B = nonsense, frame shift, splice site, or translation initiation mutation; C = multiple exon deletion. $^{*}P < 0.05$.

seizures before the age of 2 years (early-onset classical phenotype, $n = 36$ (65%; Table 2), however we also encountered patients with the onset of seizures later in life (late-onset classical phenotype, $n = 10$ (18%; Table 3). Seizure semiology was diverse. In the first year of life paroxysmal eye movements, cyanotic spells, complex absences and atonic seizures were observed. In later years, myoclonic seizures and generalized tonic–clonic attacks were seen. Seizure frequency varied from daily to sporadic attacks. Mental retardation ranged from mild ($n = 13$; 36%) to moderate ($n = 16$; 44%) or severe ($n = 6$; 17%) in patients with the early-onset classical phenotype (data on the severity of mental retardation of one patient were unavailable). In patients with the late-onset classical phenotype severity of mental retardation ranged from mild ($n = 7$; 70%) to moderate ($n = 3$; 30%). Complex movement disorders (ataxia, dystonia or chorea) were seen in 69% ($n = 25$) of the patients with the early-onset and in 90% ($n = 9$) of patients with the late-onset classical phenotype. Movement disorders were either continuous, paroxysmal or continuous with paroxysmal worsening. Data on head growth were available in 39 patients (85%) with the classical phenotype. Microcephaly was seen in 14 out of 32 patients (44%) with the early-onset, and in two out of seven patients (29%) of the patients with the late-onset classical phenotype.

Furthermore, we observed a non-classical phenotype of patients with a mental retardation and movement disorder, but without epilepsy ($n = 8$; 15%; Table 4). Mental retardation ranged from mild ($n = 4$; 50%) to moderate ($n = 2$; 25%) or severe ($n = 2$; 25%). Ataxia was seen in 75% ($n = 6$), dystonia in 50% ($n = 4$), and chorea in 38% ($n = 3$) of the patients with this non-classical phenotype. Movement disorders were continuous, paroxysmal, or continuous with paroxysmal worsening. Paroxysmal movement disorders were mostly triggered by fasting or exercise. Microcephaly was seen in 50% ($n = 4$) of the patients with the non-classical phenotype.

Finally, we identified one adult case of GLUT1 deficiency syndrome (Patient 55, the mother of Patient 24) with a normal psychomotor development and only minimal symptoms. She had one seizure when she was 2-years-old and is 'clumsy' after prolonged fasting.

Response to the ketogenic diet

A ketogenic diet was started in 37 out of 46 patients (80%) with the classical phenotype. Out of these 37 patients, 24 (62%) became totally seizure free after introduction of the diet and seizure reduction was seen in nine patients (24%). The frequency and/or severity of movement disorders was significantly reduced in 12 out of 29 patients (41%) with the classical phenotype in combination with a movement disorder. In two patients (7%) the movement disorder totally disappeared after introduction of the ketogenic diet. One of these patients (38) had paroxysmal ataxia. The other patient (33) had paroxysmal ataxia and dystonia. The diet, however, was stopped in Patient 33 because of incompliance. The diet was also stopped in two other patients with the classical phenotype, due to non-compliance.

The ketogenic diet was started in seven out of eight patients (88%) with a non-classical phenotype. Reduction of the frequency and/or severity of the movement disorder was seen in five out of seven patients (71%). The diet was stopped with one patient due to incompliance.

Subjective improvement of cognitive function after initiation of the ketogenic diet was seen in 19 out of 37 patients (51%) with a classical phenotype and in two out of seven patients (29%) with the non-classical phenotype.

Relation between genotype and phenotype

We identified specific relations between genotype and phenotype. First, type of mutation was correlated with the severity of mental retardation. Mild mental retardation was seen in 79% ($n = 15$) of the patients with a missense mutation ('type A mutation'; Table 2–4), whereas only 26% ($n = 9$) of the patients with a nonsense, frame shift, splice site, or translation initiation mutation ('type B mutation'), or multiple exon deletion ('type C mutation') had mild mental retardation ($P = 0.000$). Second, type of mutation was correlated with the presence of movement disorders. Movement disorders were less frequently seen in patients with type A mutations (63%; $n = 12$) than in patients with type B or C mutations (88%; $n = 30$; $P = 0.037$). Third, all six patients with type C mutations had the early-onset classical phenotype, whereas 38% ($n = 18$) of the patients with type B mutations had the late-onset or non-classical phenotype ($P = 0.075$). The non-classical phenotype was seen in both patients with type A (20%; $n = 4$) and patients with type B mutations (14%; $n = 4$). Patients with identical mutations were heterogeneous in phenotype and severity of mental retardation.

Relation between genotype and biochemical data

The CSF : blood glucose ratio was lower in patients with type C (0.32; SD 0.07) than in patients with type A mutations (0.40; SD 0.06; $P = 0.009$) (Fig. 2). Patients with type B mutations also had a lower CSF : blood glucose ratio (0.35; SD 0.06) than patients with type A mutations (0.40; SD 0.06; $P = 0.013$). The CSF : blood glucose ratio did not differ significantly between patients with type B or type C mutations ($P = 0.289$). The CSF glucose value was lower in patients with type C (1.48 mmol/l; SD 0.34) than in patients with type A mutations (1.96 mmol/l; SD 0.26; $P = 0.001$). The CSF glucose value was also lower in patients with type B (1.68 mmol/l; SD 0.30) than in patients with type A mutations (1.96 mmol/l; SD 0.26; $P = 0.003$). The CSF glucose did not differ significantly between patients with type B or type C mutations ($P = 0.150$). CSF lactate did not differ significantly between patients with the type A (1.10 mmol/l; SD 0.23), type B (0.97 mmol/l; SD 0.23), or type C mutations (1.20 mmol/l; SD 0.35; $P = 0.129$).

Relation between phenotype and biochemical data

Patients with the early-onset classical phenotype had a lower CSF : blood glucose ratio (0.35; SD 0.07) than patients with the late-onset classical phenotype (0.41; SD 0.07; $P = 0.009$) and

patients with the non-classical phenotype (0.41; SD 0.044; $P = 0.005$) (Fig. 2). The CSF glucose value did not differ significantly between patients with the early-onset classical (1.7 mmol/l; SD 0.35), late-onset classical (1.9 mmol/l; SD 0.30), or non-classical phenotype (1.8 mmol/l; SD 0.19; $P = 0.145$). CSF lactate did not differ significantly between patients with the early-onset classical (1.03 mmol/l; SD 0.27), late-onset classical (1.16 mmol/l; SD 0.18), or non-classical phenotype (0.94 mmol/l; SD; 0.15; $P = 0.264$).

Discussion

Our data confirm the wide clinical spectrum of GLUT1 deficiency syndrome as recently described (Brockmann, 2009). The absence of epilepsy in patients with GLUT1 deficiency syndrome is considered uncommon and has only been described in a small number of patients (Overweg-Plandsoen *et al*., 2003; Hennecke *et al*., 2005; Friedman *et al*., 2006; Klepper *et al*., 2007). We have, however, identified a relatively large number of patients in our cohort ($n = 8$; 15%) with the non-classical phenotype of mental retardation and movement disorders without epilepsy. Another non-classical phenotype of paroxysmal exercise-induced dyskinesia was recently described (Suls *et al*., 2008; Weber *et al*., 2008; Schneider *et al*., 2009). We did not identify patients with the latter phenotype in our study cohort, but we expect that with the increasing awareness of the clinical variety of GLUT1 deficiency syndrome, diagnosis will be made more frequently in patients with a non-classical phenotype.

Recognizing GLUT1 deficiency syndrome is important since it can be treated with a ketogenic diet. The ketogenic diet is a high-fat, low carbohydrate, normocaloric diet which results in permanent ketosis and provides the brain with an alternative fuel (Klepper *et al*., 2004). It is currently believed that the ketogenic diet should be initiated as soon as possible and should be maintained at least until adolescence (Klepper *et al*., 2005; Brockmann, 2009). In our study cohort, the ketogenic diet was effective in most of the patients with epilepsy (86%) and also reduced movement disorders significantly in 48% of the patients with the classical phenotype and 71% of the patients with a non-classical phenotype. Effectiveness of the ketogenic diet in a series of drug-resistant epileptic patients with GLUT1 deficiency syndrome has previously been described (Klepper *et al*., 2005), but improvement of movement disorders in patients with a non-classical phenotype has only been observed in case reports (Friedman *et al*., 2006; Brockmann, 2009). Our study underlines the importance of initiating a ketogenic diet, not only in patients with epilepsy, but also in patients with movement disorders without epilepsy. Although subjective improvement of cognitive function, behaviour and alertness was reported in a substantial number of our patients, no objective data exist on the effect of the ketogenic diet on cognitive function. Prospective follow-up studies with standardized neurocognitive tests are needed.

The delay in diagnosing GLUT1 deficiency syndrome is considerable. Patients with the classical phenotype were diagnosed at an average of 6.6 years after the onset of seizures (range 1 month–16 years) (Tables 2 and 3). Our study demonstrates that a lumbar puncture provides the diagnostic clue to GLUT1 deficiency syndrome and can thereby dramatically reduce diagnostic delay to allow early start of the ketogenic diet. All GLUT1 deficiency syndrome patients in our cohort had a low CSF glucose concentration (<2.5 mmol/l) and all but one patient had a low CSF : blood glucose ratio (<0.50). Differentiation with other causes of a low CSF glucose and low CSF : blood glucose ratio (bacterial, fungal, or protozoal meningitis and subarachnoidal haemorrhage) can be made not only on the clinical symptoms or CSF cultures, but also on CSF lactate concentrations. Neurons can use lactate as an alternative fuel under circumstances of hypoglycaemia. A low CSF lactate in most patients with GLUT1 deficiency syndrome is therefore thought to reflect the glucose shortage in the brain (Wang *et al.*, 2006). CSF lactate was below 1.6 mmol/l in all our patients and below 1.3 mmol/l in 78% of the patients, whereas CSF lactate is elevated (>2.0 mmol/l) in meningitis and subarachnoidal haemorrhage (Cameron *et al.*, 1993). Furthermore, CSF cell count and protein are normal in GLUT1 deficiency syndrome. We therefore advise to perform a lumber puncture in patients with drug-resistant epilepsy and mental retardation, as well as in patients with unexplained movement disorders, especially if movement disorders worsen or occur after fasting or exercise. Patients with paroxysmal exercise-induced dyskinesia due to GLUT1 deficiency syndrome have been observed in the absence of mental retardation, migraine or epilepsy (Schneider *et al.*, 2009). Notably, in patients with GLUT1 deficiency syndrome with this non-classical phenotype of familial exercise-induced dyskinesia the CSF is less sensitive for diagnosing GLUT1 deficiency syndrome, as normal values for CSF : blood glucose ratio (0.47–0.60; mean 0.52) and CSF glucose (1.8–3.6 mmol/l; mean 2.4 mmol/l) can be found (Suls *et al.*, 2008).

Our results demonstrate that GLUT1 deficiency syndrome is caused by (partial) deletion of the *SLC2A1* gene in at least 10% of the patients with GLUT1 deficiency syndrome confirmed by DNA analysis. To date, only five GLUT1 deficiency syndrome patients with a large scale deletion have been reported (De Vivo *et al.*, 1991; Seidner *et al.*, 1998; Wang *et al.*, 2000; Vermeer *et al.*, 2007). Perhaps, multiple exon deletions of a *SLC2A1* allele are under-diagnosed at the DNA level as a cause of GLUT1 deficiency syndrome. We advise using multiplex ligation-dependent probe amplification in every patient suspected of GLUT1 deficiency syndrome, especially in patients with an early-onset classical phenotype, since all patients identified with a multiple exon deletion had this phenotype. By performing multiplex ligation-dependent probe amplification in combination with Sanger-based automated sequencing we were able to confirm suspicion of the clinical and/or biochemical diagnosis of GLUT1 deficiency syndrome at the DNA level in 42% of all requests for DNA analysis of the *SLC2A1* gene. Regulatory sequences of the *SLC2A1* gene, e.g. promoter sequences and/or sequences deep in introns are not included in the molecular diagnostic strategy. Possible mutations in these regions thus may not have been uncovered.

We identified 13 novel missense mutations in the *SLC2A1* gene. Eleven of these missense mutations were found in patients with the classical phenotype of GLUT1 deficiency syndrome of epilepsy and mental retardation in combination with movement disorders and a characteristic CSF pattern, which would support the

presence of a pathogenic GLUT1 allele. Since just a single DNA variant was identified in the coding region in these cases, this strongly suggests the potential pathogenic nature of these mutations. Furthermore, the position of the mutations in the GLUT1 protein indicates the pathogenicity of the individual mutations. A novel missense mutation was identified in exon 2 (p.Asn34Tyr). Asn34 is the first amino acid in the large extracellular loop connecting transmembrane domains 1 and 2 (Hruz and Mueckler, 2001). It is a conserved residue in most of the GLUT subclasses (Joost and Thorens, 2001) and therefore appears to be a crucial residue for glucose transport. Three other patients have previously been reported with a missense mutation at this location (Wang *et al.*, 2000; Overweg-Plandsoen *et al.*, 2003; Klepper *et al.*, 2005). Two of our patients had the novel missense mutation p.Ala155Val. Ala 155 is part of the transmembrane segment 5, which is predicted to be part of the central channel of GLUT1 and is therefore critical for glucose transport (Salas-Burgos *et al.*, 2004). The alanine residue is part of an evolutionary conserved amino-acid stretch (in mouse, rat, dog, chicken, xenopus and fugus). The novel missense mutations p.Arg212His and p.Arg212Cys were identified in the Patients 7 and 8. Arg212 is part of the large cytoplasmic loop between helices 6–7. Another novel missense mutation was located in the cytoplasmic loop between helices 6–7 (p.Arg223Trp). This mutation is considered pathogenic, since it is not documented in the Single Nucleotide Polymorphism database and has not been identified in more than 100 control alleles. The 'Sorting Intolerant from Tolerant' (SIFT) program predicts that the protein will not tolerate the amino-acid change. Several other crucial residues have previously been identified in this loop [Arg218 (Klepper *et al*., 2005), Lys256 (Wang *et al.*, 2000), Ala275 (Weber *et al*., 2008)]. We identified a novel mutation in exon 8 (p.Glu329Gln). Glu329 is located at the cytoplasmic loop between helices 8–9. Mutation of Glu345 in the cytoplasmic loop between helices 8–9 in GLUT4, which corresponds with Glu329 in GLUT1, has been shown to lock the transporter in an inward-facing confirmation (Schurmann *et al.*, 1997). Another novel missense mutation was found in exon 8 (Arg333Gln). Arg 333 is part of the highly conserved amino-acid motif Arg-X-Gly-Arg-Arg between helix 8–9. The positive charges in the motif form critical local cytoplasmic anchor points involved in determining the membrane topology in GLUT1. These Arg-X-Gly-Arg-Arg motifs are conserved in many of the members of the major facilitator superfamily. Removal of the positive charges in these motifs causes an aberrant flipping of the corresponding cytoplasmic loop into the exoplasmic compartment (Sato and Mueckler, 1999). Two novel missense mutations were detected in exon 9 (p.Gly382Asp and p.Ala405Asp). Gly382 is located in helix 10 and concerns an evolutionary high conserved residue. Transmembrane segment 10 forms a part of the central aqueous channel. Expression of a Gly382Asp mutant in *X. laevis* oocytes showed abolished glucose transport capacity and reduced GLUT1 expression on the cell membrane (Mueckler and Makepeace, 2002). Ala405 is located in transmembrane segment 11, which is also predicted to be part of the central aqueous channel. Cysteine-scanning mutagenesis of transmembrane segment 11 showed that Ala405 had significantly increased specific activity relative to the cysteine-less protein (Hruz and Mueckler, 2000). Cysteine and alanine are both amino acids with non-polar side chains. Our patient, however, had alanine replaced by aspartic acid, an amino acid with a polar side chain and negative side chain charge, which changes the hydropathy and therefore is likely to disrupt glucose transport across the aqueous central channel. A novel heterozygous mutation was detected in Patient 10 in exon 10 (p.Pro485Leu). The mutation was located in the cytoplasmic carboxyl tail of the GLUT1 protein and was proven to be *de novo*.

We cannot completely prove the pathogenic nature of two novel missense mutations. Missense mutation p.Arg153His was identified in a patient with a non-classical phenotype of mild mental retardation, continuous ataxia and paroxysmal dystonia and choreoathetosis. The DNA of the parents of this patient was not investigated. The characteristic CSF pattern with low CSF glucose, low CSF : blood glucose ratio and low CSF lactate, however, supports the pathogenicity of this mutation. Furthermore, Arg153 is positioned in the cytoplasmic loop between helix 3 and 4 and is a crucial residue in a conserved domain. Arg153 is one of the known mutation hotspots in GLUT1 deficiency syndrome (Klepper and Leiendecker, 2007). Patient 57 was identified with the novel missense mutation p.Val303Leu. Val303is located in the exofacial loop between helix 7–8. This patient, unfortunately, was lost to follow-up and clinical data were unavailable. The DNA of the parents of the patient was not investigated. The pathogenic nature of this mutation cannot, therefore, be supported.

Autosomal dominant transmission of GLUT1 deficiency syndrome has only been described in a few families with paroxysmal exercise-induced dyskinesia (Suls *et al*., 2008; Weber *et al*., 2008) and some families with the classical phenotype of GLUT1 deficiency syndrome (Brockmann *et al.*, 2001; Klepper *et al.*, 2001). We identified autosomal dominant transmission of GLUT1 deficiency syndrome in four families (nine patients). Although this finding confirms that GLUT1 deficiency syndrome is caused by *de novo* mutations of the *SLC2A1* gene in the majority of patients, it emphasizes on the other hand the importance of further clinical and genetic investigation of family members of GLUT1 deficiency syndrome patients.

The genotypic and phenotypic variability among patients with GLUT1 deficiency syndrome is substantial. Our relatively large series of 57 patients with GLUT1 deficiency syndrome allowed, for the first time, an analysis of genotype–phenotype correlations. It has previously been speculated that large-scale deletions, nonsense, frame shift and splice-site mutations result in 50% loss of the GLUT1 protein and are associated with the moderate, classical phenotype of GLUT1 deficiency syndrome. Heterozygous missense mutations with 50–75% residual function are thought to be associated with mild phenotypes. Missense mutations that result in more than 75% residual transport capacity are speculated to be associated with a minimal phenotype with symptoms related to environmental factors, such as fasting or the use of caffeine (Wang *et al.*, 2005). To allow comparison of the effect of different types of mutation on the phenotype, we have clustered mutations that are likely to give a large disruption of glucose transport into brain (nonsense, frame shift, splice site and translation initiation mutations ('type B mutations') and multiple exon deletions ('type C mutations'), and compared them with missense mutations

('type A mutations'; Table 2–4). Our data confirm that type of mutation is related to the phenotype. Movement disorders were more frequently seen in patients with type B or C mutations, than in patients with type A mutations. Furthermore, type of mutation was related to the severity of mental retardation. Mild retardation was found more often in patients with type A than in patients with type B or C mutations. However, severe mental retardation was found in a few patients with type A mutations and, in contrast, a mild retardation in some patients with a type B or C mutations. Additionally, patients with the same mutation displayed a heterogeneous range of type and severity of phenotype. Even within families with autosomal dominant transmission of GLUT1 deficiency syndrome, phenotypic diversity was significant, which suggests that secondary genes and other proteins may be involved in glucose transport. Another explanation for the phenotypic diversity is a modulating effect of DNA variants regulatory elements of the wild-type GLUT1 allele that may modulate the expression level of the GLUT1 transporter. This phenotypic diversity within families with autosomal transmission of GLUT1 deficiency syndrome has important implications for genetic counselling. It should be emphasized that although a patient has very few symptoms it is possible that a future child with the same mutation will be severely affected. On the other hand, the argument may be reversed in case of a more severely affected patient.

For the first time, genotype could be correlated with CSF parameters (Fig. 2A–C). Patients with type B or C mutations had lower CSF : blood glucose ratios and lower CSF glucose than patients with type A mutations. This suggests that the residual GLUT1 activity in the blood–brain barrier correlates with the CSF : blood glucose ratio as well as the CSF glucose. In addition, the CSF : blood glucose ratio was correlated with phenotype (Fig. 2D–F). The early-onset classical phenotype was correlated with lower CSF : blood glucose ratios than the late-onset classical phenotype and the non-classical phenotype. This suggests that the degree of disruption of glucose transport into the brain is an indicator of the type and severity of clinical symptoms in GLUT1 deficiency syndrome. In the individual patient, however, a very low CSF : blood glucose ratio was not always related to a more severe phenotype. Apparently, the CSF : blood glucose ratio does not (solely) reflect the severity of the symptoms in GLUT1 deficiency syndrome. A possible explanation for a very low CSF : blood glucose ratio in patients with a relatively mild phenotype is the order in which the lumbar and vein puncture have been performed. If blood samples are taken after the lumbar puncture is performed, the blood glucose can be raised due to stress and this will lower the calculated CSF : blood glucose ratio. Furthermore, CSF and blood glucose can fluctuate in the individual patient due to fasting and stress.

In conclusion, GLUT1 deficiency syndrome is a treatable disorder of glucose transport into the brain caused by a variety of mutations in the *SLC2A1* gene, which lead to a neurological disorder with large phenotypic diversity, including a substantial number of patients without epilepsy. A lumbar puncture should be performed in every patient suspected of GLUT1 deficiency syndrome, as the simple measurement of CSF glucose is an inexpensive, widely available method to rapidly provide a sensitive marker for selecting patients for mutation analysis of the *SLC2A1* gene and early introduction of the ketogenic diet.

Funding

W.G. Leen was supported by The Netherlands Organisation for Scientific Research (NWO; www.nwo.nl).

References

Baldwin SA. Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. Biochim Biophys Acta 1993; 1154: 17–49.

Barros LF, Bittner CX, Loaiza A, Porras OH. A quantitative overview of glucose dynamics in the gliovascular unit. Glia 2007; 55: 1222–37.

Bertsche A, Santer R, Vater D, Ebinger F, Rating D, Wolf N. GLUT1 deficiency in a child with a movement disorder (Abstract). Neuropediatrics 2009; 40.

Brockmann K. The expanding phenotype of GLUT1-deficiency syndrome. Brain Dev 2009; 31: 545–52.

Brockmann K, Wang D, Korenke CG, Von Moers A, Ho YY, Pascual JM, et al. Autosomal dominant glut-1 deficiency syndrome and familial epilepsy. Ann Neurol 2001; 50: 476–85.

Cameron PD, Boyce JM, Ansari BM. Cerebrospinal fluid lactate in meningitis and meningococcaemia. J Infect 1993; 26: 245–52.

De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. N Engl J Med 1991; 325: 703–9.

Friedman JR, Thiele EA, Wang D, Levine KB, Cloherty EK, Pfeifer HH, et al. Atypical GLUT1 deficiency with prominent movement disorder responsive to ketogenic diet. Mov Disord 2006; 21: 241–5.

Fukumoto H, Seino S, Imura H, Seino Y, Bell GI. Characterization and expression of human HepG2/erythrocyte glucose-transporter gene. Diabetes 1988; 37: 657–61.

Hennecke M, Wang D, Korinthenberg R, Pascual J, Yang H, Engelstad K, et al. GLUT1 deficiency syndrome with ataxia, acquired microcephaly and leukoencephalopathy in monozygotic twins (Abstract). Neuropediatrics 2005; 36: 140.

Hruz PW, Mueckler MM. Cysteine-scanning mutagenesis of transmembrane segment 11 of the GLUT1 facilitative glucose transporter. Biochemistry 2000; 39: 9367–72.

Hruz PW, Mueckler MM. Structural analysis of the GLUT1 facilitative glucose transporter (review). Mol Membr Biol 2001; 18: 183–93.

Ito S, Oguni H, Ito Y, Ishigaki K, Ohinata J, Osawa M. Modified Atkins diet therapy for a case with glucose transporter type 1 deficiency syndrome. Brain Dev 2008; 30: 226–8.

Joost HG, Thorens B. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol Membr Biol 2001; 18: 247–56.

Joshi C, Greenberg CR, De Vivo D, Dong W, Chan-Lui W, Booth FA. GLUT1 deficiency without epilepsy: yet another case. J Child Neurol 2008; 23: 832–4.

Klepper J, Diefenbach S, Kohlschutter A, Voit T. Effects of the ketogenic diet in the glucose transporter 1 deficiency syndrome. Prostaglandins Leukot Essent Fatty Acids 2004; 70: 321–7.

Klepper J, Engelbrecht V, Scheffer H, van der Knaap MS, Fiedler A. GLUT1 deficiency with delayed myelination responding to ketogenic diet. Pediatr Neurol 2007; 37: 130–3.

Klepper J, Garcia-Alvarez M, O'Driscoll KR, Parides MK, Wang D, Ho YY, et al. Erythrocyte 3-O-methyl-D-glucose uptake assay for diagnosis of glucose-transporter-protein syndrome. J Clin Lab Anal 1999a; 13: 116–21.

Klepper J, Leiendecker B. GLUT1 deficiency syndrome–2007 update. Dev Med Child Neurol 2007; 49: 707–16.

Klepper J, Scheffer H, Leiendecker B, Gertsen E, Binder S, Leferink M, et al. 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 2005; 36: 302–8.

Klepper J, Voit T. Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome: impaired glucose transport into brain—a review. Eur J Pediatr 2002; 161: 295–304.

Klepper J, Wang D, Fischbarg J, Vera JC, Jarjour IT, O'Driscoll KR, et al. Defective glucose transport across brain tissue barriers: a newly recognized neurological syndrome. Neurochem Res 1999b; 24: 587–94.

Klepper J, Willemsen M, Verrips A, Guertsen E, Herrmann R, Kutzick C, et al. Autosomal dominant transmission of GLUT1 deficiency. Hum Mol Genet 2001; 10: 63–8.

Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, et al. Sequence and structure of a human glucose transporter. Science 1985; 229: 941–5.

Mueckler M, Makepeace C. Analysis of transmembrane segment 10 of the Glut1 glucose transporter by cysteine-scanning mutagenesis and substituted cysteine accessibility. J Biol Chem 2002; 277: 3498–503.

Mueckler M, Makepeace C. Model of the exofacial substrate-binding site and helical folding of the human Glut1 glucose transporter based on scanning mutagenesis. Biochemistry 2009; 48: 5934–42.

Overweg-Plandsoen WC, Groener JE, Wang D, Onkenhout W, Brouwer OF, Bakker HD, et al. GLUT-1 deficiency without epilepsy–an exceptional case. J Inherit Metab Dis 2003; 26: 559–63.

Roulet-Perez E, Ballhausen D, Bonafe L, Cronel-Ohayon S, Maeder-Ingvar M. Glut-1 deficiency syndrome masquerading as idiopathic generalized epilepsy. Epilepsia 2008; 49: 1955–8.

Salas-Burgos A, Iserovich P, Zuniga F, Vera JC, Fischbarg J. Predicting the three-dimensional structure of the human facilitative glucose transporter glut1 by a novel evolutionary homology strategy: insights on the molecular mechanism of substrate migration, and binding sites for glucose and inhibitory molecules. Biophys J 2004; 87: 2990–9.

Sato M, Mueckler M. A conserved amino acid motif (R-X-G-R-R) in the Glut1 glucose transporter is an important determinant of membrane topology. J Biol Chem 1999; 274: 24721–5.

Schneider SA, Paisan-Ruiz C, Garcia-Gorostiaga I, Quinn NP, Weber YG, Lerche H, et al. GLUT1 gene mutations cause sporadic paroxysmal exercise-induced dyskinesias. Mov Disord 2009; 24: 1684–8.

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002; 30: e57.

Schurmann A, Doege H, Ohnimus H, Monser V, Buchs A, Joost HG. Role of conserved arginine and glutamate residues on the cytosolic surface of glucose transporters for transporter function. Biochemistry 1997; 36: 12897–902.

Seidner G, Alvarez MG, Yeh JI, O'Driscoll KR, Klepper J, Stump TS, et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain barrier hexose carrier. Nat Genet 1998; 18: 188–91.

Shows TB, Eddy RL, Byers MG, Fukushima Y, Dehaven CR, Murray JC, et al. Polymorphic human glucose transporter gene (GLUT) is on chromosome 1p31.3—p35. Diabetes 1987; 36: 546–9.

Slaughter L, Vartzelis G, Arthur T. New GLUT-1 mutation in a child with treatment-resistant epilepsy. Epilepsy Res 2009; 84: 254–6.

Suls A, Dedeken P, Goffin K, Van Esch H, Dupont P, Cassiman D, et al. Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in SLC2A1, encoding the glucose transporter GLUT1. Brain 2008; 131: 1831–44.

Takahashi S, Ohinata J, Suzuki N, Amamiya S, Kajihama A, Sugai R, et al. Molecular analysis and anticonvulsant therapy in two patients with glucose transporter 1 deficiency syndrome: a successful use of zonisamide for controlling the seizures. Epilepsy Res 2008; 80: 18–22.

Ticus I, Cano A, Villeneuve N, Milh M, Mancini J, Chabrol B. [GLUT-1 deficiency syndrome or De Vivo disease: a case report]. Arch Pediatr 2008; 15: 1296–9.

Vannucci SJ, Maher F, Simpson IA. Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia 1997; 21: 2–21.

Vermeer S, Koolen DA, Visser G, Brackel HJ, Van der Burgt I, De Leeuw N, et al. A novel microdeletion in 1(p34.2p34.3), involving the SLC2A1 (GLUT1) gene, and severe delayed development. Dev Med Child Neurol 2007; 49: 380–4.

Wang D, Kranz-Eble P, De Vivo DC. Mutational analysis of GLUT1 (SLC2A1) in Glut-1 deficiency syndrome. Hum Mutat 2000; 16: 224–31.

Wang D, Pascual JM, Yang H, Engelstad K, Jhung S, Sun RP, et al. Glut-1 deficiency syndrome: clinical, genetic, and therapeutic aspects. Ann Neurol 2005; 57: 111–8.

Wang Y, Zhou J, Minto AW, Hack BK, Alexander JJ, Haas M, et al. Altered vitamin D metabolism in type II diabetic mouse glomeruli may provide protection from diabetic nephropathy. Kidney Int 2006; 70: 882–91.

Weber YG, Storch A, Wuttke TV, Brockmann K, Kempfle J, Maljevic S, et al. GLUT1 mutations are a cause of paroxysmal exertion-induced dyskinesias and induce hemolytic anemia by a cation leak. J Clin Invest 2008; 118: 2157–68.

Zorzi G, Castellotti B, Zibordi F, Gellera C, Nardocci N. Paroxysmal movement disorders in GLUT1 deficiency syndrome. Neurology 2008; 71: 146–8.