GLUT1 DEFICIENCY SYNDROME:
DIAGNOSIS AND TREATMENT
IN OUR ITALIAN CASE STUDY

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Irene
GLUT1 DS
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INTRODUCTION

Glucose is the essential substrate for brain energy metabolism. In the resting state, the adult brain can consume up to 25% of the body’s total glucose supply (Klepper J, 2008), while in infants and children it can use as much as 80% (Cremer JE, 1982).

The diffusion of this essential fuel across the blood-brain barrier is facilitated by glucose transporter type 1 (GLUT1). In the brain, GLUT1 interacts with other specific GLUT1 isoforms mediating glucose transport into astrocytes and neurons. GLUT1 deficiency syndrome (GLUT1DS) results from impaired glucose transport into the brain (Klepper J & Voit T, 2002).

The classic GLUT1DS patient presents with drug-resistant infantile seizures, developmental delay, acquired microcephaly, hypotonia, spasticity, and a complex movement disorder consisting of ataxia and dystonia (Klepper J & Leiendecker B, 2007).

Recently the clinical spectrum of GLUT1DS has been broadened to include developmental delay, epilepsy and/or movement disorders (Brockmann K, 2009), as well as familial and sporadic paroxysmal exercise-induced dyskinesia with or without epilepsy (Schneider SA et al., 2009). There are also varying degrees of cognitive impairment associated with dysarthria, dysfluency, and expressive language deficits. In most patients, the cerebrospinal fluid (CSF)-to-blood glucose ratio is below 0.50, and CSF lactate is low to normal.

A diagnosis of GLUT1DS can be confirmed by molecular analysis of the SLC2A1 gene, while analysis of glucose uptake into erythrocytes can confirm the impaired GLUT1 function. Early diagnosis is critical because it allows prompt initiation of treatment with a ketogenic diet (KD), which is a high fat, low carbohydrate diet that mimics the metabolic state of fasting. Since ketone bodies use another transporter to enter the central nervous system, they can provide the brain with an alternative source of fuel, thereby effectively correcting the impaired brain energy metabolism (Leen WG et al, 2010) and reducing the frequency of the seizures and the severity of the dystonic movement disorder.

The normal rate of cerebral oxygen consumption is low during the fetal and perinatal periods; it then increases in a linear fashion to peak at the age 3 of years, thereafter remaining high until adolescence.
when it shows a gradual decline (Graham JM, 2012). Therefore, childhood is the critical period for treatment of GLUT1DS: early diagnosis is crucial for effective KD treatment.

**Figure 2**

*Normal glucose pathway across the cell*
MOLECULAR BASIS

GLUT1 is a member of the GLUT family of facilitative glucose transporters, which comprises 13 proteins (gene symbol SLC2A, protein symbol GLUT). Cloned and sequenced in 1985, GLUT1/SLC2A1 (OMIM 138140) was the first gene of this family to be identified (Mueckler M et al., 1985). Located on the short arm of chromosome 1 (1p34.2), this gene is 35 kb long and consists of 10 exons. GLUT1 is constitutively expressed in most tissues and selectively expressed in erythrocytes, brain microvessels and astroglia. It exists in two molecular weight forms (45 and 55 kDa), which differ only in the extent of glycosylation of the protein (Salas-Burgos A et al., 2004). The 45 kDa form is detected in most cells including astrocytes and may be responsible for basal glucose uptake by cells. The 55 kDa form is found predominantly in the endothelial cells of brain microvessels and erythrocytes, where it is the principal glucose transporter (Brockmann K, 2009). If there is a deficiency of GLUT1, glucose cannot pass from the blood into the brain, and this causes a dysfunction in the central nervous system.

Human and animal data suggest that there is only a narrow margin of safety for glucose transport across the blood-brain barrier to meet the needs of brain metabolism and cerebral function. A milder clinical phenotype with intermittent symptoms (epilepsy, dyskinesias, ataxia) may be predicted in the presence of a 25%-35% reduction in GLUT1 transporter function (Rotstein M et al., 2010), while a more severe phenotype is likely to result from reductions in the order of 40%-75% (Yang H et al., 2011).

Most detected SCL2A1 mutations are de novo; in familial cases the condition is inherited as an autosomal dominant trait with complete penetrance. Detection of the disease-causing mutation in an asymptomatic parent implies a mosaic state. A case of autosomal recessive transmission has also recently been described (Klepper J et al., 2009). All detected mutations are heterozygous; indeed, homozygous GLUT1 mutations are presumably lethal in utero (Perez-Duenas B et al., 2009).

Patients with missense mutations generally present with moderate to mild symptoms, but no clear-cut phenotype-genotype correlations have been established. Indeed, patients sharing identical mutations often do not have identical clinical manifestations, which suggests that there are additional mechanisms at work, such as disease-modifying genes and proteins that alter phenotype and potentially contribute to the pathophysiology of this complex entity (Nickels K & Wirrell, 2010). Leen (Leen et al., 2010) studied 57 patients, trying to identify a specific relationship between genotype and phenotype. They found that mild mental retardation was more often present in patients with a missense mutation (Type A), and
movement disorders in those with translation initiation mutations (Type B) or multiple exon deletions (Type C). However, severe mental retardation was found to be present in a few Type A patients and mild mental retardation in some Type B or C patients. Additionally, patients with the same mutation displayed phenotypic heterogeneity, in terms of range of clinical expression and severity. It is possible that other secondary genes or other proteins are involved in glucose transport, which might explain the phenotypic diversity of this disease (Verrotti A et al., 2012).

GLUT1 deficiency syndrome may also occur as a part of broader genetic syndromes, as seen in microdeletion syndromes involving the SLC2A1 gene (Aktas D et al., 2010).

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**Figure 3**

Homology-based modelling of GLUT1 structure.

*a*, A structural model of GLUT1 is generated on the basis of XylE structure and sequence conservation. Invariant and conserved residues are coloured magenta and dark green, respectively. *b*, Predicted secondary structural elements of GLUT1. Polar and aromatic residues predicted to be involved in D-glucose binding are shaded red and yellow, respectively. The residues whose mutations were found in GLUT1 deficiency syndrome are shaded purple and blue for invariant and variant residues, respectively. *c*, Mapping of the disease-related residues on the structural model of GLUT1. Invariant and variant residues in XylE and GLUT1–4 are labelled red and blue, respectively. *d*, Mutation of Arg 133 or Arg 341 in XylE, which correspond to Arg 126 and Arg 333 of GLUT1 (highlighted in magenta in c), led to abrogation of D-xylose transport in a liposome-based counterflow assay. 

CLINICAL PHENOTYPES

- Classical phenotype

GLUT1 deficiency syndrome was first described in 1991 as an early-onset childhood epileptic encephalopathy (De Vivo DC et al., 1991). This phenotype, now defined “classical”, was rapidly expanded to include epileptic encephalopathy with different seizure types, developmental delay, acquired microcephaly, complex movement disorders (variable combinations of ataxia, dystonia and spasticity), and paroxysmal events. Seizures were initially described as brief, subtle myoclonic limb jerking with alternating staring and eye-rolling, unresponsiveness, hypotonia and head bobbing. Now, also generalized seizures of all types (absences, generalized tonic-clonic, myoclonic, atonic, etc.) are considered features of the classical phenotype. The movement disorders presented differ from patient to patient, but most frequently consist of ataxia and spasticity. Paroxysmal events of possibly non-epileptic origin include intermittent ataxia, periodic confusion, periodic weakness, periodic limb paralysis, recurrent headaches, and intermittent sleep disturbances. The clinical severity of the classical phenotype varies, ranging from mild motor and cognitive dysfunction between epileptic attacks to severe neurological disability, with some patients never achieving the ability to speak or walk unsupported (Brockmann K, 2009). In most patients with the classical phenotype neurological symptoms are not influenced by fasting or food intake (De Vivo DC et al., 2002).

- Non-classical phenotypes

Several clinical variants of GLUT1DS have been reported over the two decades since the first description of the classical phenotype. The most cited in the literature is Brockmann’s classification (Brockmann K, 2009), consisting of: Patients with (i) carbohydrate-responsive symptoms, characterized by a correlation between fasting and neurological deterioration including seizure frequency, with (ii) predominant ataxia or dystonia, but without seizures, and with (iii) paroxysmal exertion-induced dyskinesia and seizures. However, over the years numerous atypical clinical manifestations have been described, such as paroxysmal exertion-induced dyskinesia (PED) with (Zorzi et al., 2008) or without seizures (Suls A et al., 2008; Weber YG et al., 2008; Schneider SA et al., 2009), choreoathetosis (Friedman JR et al., 2006), alternating hemiplegia (Rostein M et al., 2009), and other paroxysmal events (Urbizu et al., 2010) such as intermittent ataxia, dystonia, migraine.
- Current state of the art

At the current state of the art in understanding of GLUT1DS, classifying the disease phenotype as “classical” or “non-classical” seems to be of limited clinical utility. It should be considered, rather, as a broad clinical spectrum in which we can observe with various levels of severity:

Intellectual impairment:

Intellectual impairment in patients with GLUT1DS varies widely and may include: language delay, expressive language difficulties possibly associated with dysarthria, learning difficulties, and mild, moderate or severe cognitive delay, but without a significant neuropsychological profile. In our experience, cognitive impairment is often proportional to the severity of other symptoms; indeed IQ is usually normal in patients with minimal symptoms.

Leen, in his sample (Leen WG et al. 2010) of 57 patients, found mental retardation (from mild to severe) in almost all the patients with a severe phenotype (46 out of 48), in half of those with a mild phenotype (4 out of 8), and in neither of the two patients with minimal symptoms.

Other psychiatric symptoms, such as behavioural difficulties, depression, and attention deficit hyperactivity disorder, are only sporadically described (Tzadok M et al., 2012).

Acquired microcephaly:

This is a frequent but not essential component of the clinical spectrum.

Acquired microcephaly was mainly observed in classical phenotype patients (Klepper J et al., 1998). Adult cases with “minimal symptoms” do not present microcephaly, whereas it can be found in around 40% of patients with mild phenotypes and in around 50% of those with a severe phenotype (classical De Vivo syndrome) (Leen et al., 2010).

Epilepsy:

GLUT1 deficiency syndrome should be considered in the differential diagnosis of any form of intractable epilepsy (Klepper J, 2012). Patients usually develop seizures in infancy and early childhood.

In infants, seizures are described as brief, subtle myoclonic limb jerks with alternating staring and eye-rolling, sudden-onset pallor, a dazed expression, horizontal roving eye movements, unresponsiveness, hypotonia and head bobbing. The EEG usually shows multifocal spike discharges.

Pong (Pong AW et al., 2012) recently reviewed 87 patients with Glut 1 DS and she noticed that Seventy-eight (90%) had epilepsy with average onset at 8 months. Seizures were mixed in: generalized tonic-clonic (53%), absence (49%), complex partial (37%), myoclonic (27%), drop (26%), tonic (12%), simple
In childhood, seizures are frequently myoclonic and generalized (Klepper J et al., 1998); myoclonic astatic epilepsy (MAE) is a commonly reported form (Mullen SA et al., 2011; Vieker S et al., 2012). This finding could be linked to brain maturation; indeed, as the brain matures, seizures become synchronized and manifest clinically as generalized events associated with 3-4 Hz spike-and-wave discharges.

Some authors have described forms of epilepsy that are particularly responsive to carbohydrate intake (Brockmann K, 2009).

However, it is important to stress that forms described as MAE and caused by SLC2A1 mutations cannot be considered “typical”: GLUT1DS was present in only 4 out of 84 MAE probands (5%) investigated by Mullen (Mullen SA et al., 2011) and it is important to underline that two of these patients also presented PED in childhood, and another patient presented ataxia, dysarthric speech and deceleration of head growth.

Idiopathic generalized epilepsies (IGEs), not usually drug-responsive, have also been previously described. More than 10% of early-onset (Mullen SA et al., 2010, Suls A et al., 2009) or myoclonic (Gokben et al., 2011) absence epilepsies are caused by mutations of the SLC2A1 gene.

Recently, a very interesting article by Arsov and Sheffer (Arsov T et al., 2012) showed that mutations of SLC2A1 are responsible for approximately 1% of all IGEs. The authors analyzed a total of 504 probands with IGEs (juvenile myoclonic epilepsy -32%-, juvenile absence epilepsy -21%-, childhood absence epilepsy -31%-, generalized tonic-clonic seizures -13%-, and unspecified forms of IGE -2%-) and 470 controls. In total, seven of the 504 probands and none of the 470 controls had mutations demonstrating a functional effect on the protein product. The cases described had a history of IGEs, with seizures responding well to antiepileptic drugs (AEDs), and normal intellectual outcome, although closer analysis of the patients’ clinical records revealed cases with atypical forms of juvenile myoclonic epilepsy who became seizure-free on oxcarbazepine, and cases with associated exercise-induced movement disorders.

Prior to this study, a GLUT1DS family with PED and IGEs had already been described (Afawi Z et al., 2010). This family showed adolescence/adult onset of epilepsy but, oddly and atypically, clear and definite EEG focalization.

Tzadok (Tzadok M et al., 2013) recently reported a series of eight patients with supposed IGEs but quite atypical features, namely: absence epilepsy without myoclonias but with 3/sec spike-and-wave discharges interspersed with polyspike waves, myoclonic absences associated with ataxia and mild
mental retardation, and aspecific seizures with PED. A quarter of these patients with epilepsy were found to have IGEs and the authors showed GLUT1 deficiency to be an important cause of IGEs.

At present, GLUT1 deficiency should be considered the most common known monogenic cause of atypical IGEs, and this raises the question of whether abnormalities in glucose delivery form part of a general, shared mechanism in IGEs.

Movement disorders:
Subjects with GLUT1DS commonly present complex movement disorders, which can be characterized by ataxia, dystonia, and chorea. These disorders can be continuous and/or paroxysmal and fluctuating in response to different environmental stressors (Leen WG et al., 2010). The most frequent stressors are fasting, infections, prolonged exercise, and anxiety or other emotions. Pons (Pons R et al., 2010) listed the most frequent movement disorders in 57 GLUT1DS patients: gait disturbances such as ataxia with/without spasticity (89%), action limb dystonia (86%), chorea (75%), cerebellar action tremor (70%), non-epileptic paroxysmal events (28%), dyspraxia (21%), and myoclonus (16%).

Epilepsy and movement disorders can occur either separately or in combination (Veggiotti P et al., 2010). Severe motor disorders, including dyskinesia, ataxia, chorea and spasticity, associated with severe mental retardation have been observed in the absence of seizures (Verrotti E et al., 2012).

Paroxysmal choreoathetosis with spasticity, previously known as dystonia type 9 (DYT9), and PED, previously known as dystonia type 18 (DYT 18), are now recognised to be part of the phenotypic spectrum of GLUT1DS (Suls A et al., 2008; Weber YG et al., 2008; Klepper J, 2009).

Other paroxysmal events that can be observed include weakness, lethargy, somnolence, sleep disturbances, migraines (Weber YG et al., 2008), writer's cramp, parkinsonism, dyspraxia, and non-kinesigenic dyskinesia (Overweg-Plandsoen WC et al., 2003; Pérez-Dueñas B et al., 2009).

Hemolytic anemia:
GLUT1 is the primary mediator of glucose transport across the endothelium of the blood-brain barrier, into and out of astrocytes, and into erythrocytes. However, there are rare reports of a correlation between anemia and GLUT1: Weber (Weber YG et al. 2008), identified a mutation (Q282_S285del) in the pore region of GLUT1 in a family with PED and hemolytic anemia; functional studies were performed in erythrocytes, and the mutation was found to explain the observed permanent cation leak responsible for the hemolytic anemia in this family.

Flatt (Flatt JF et al., 2011) suggested that two cases of stomatin-deficient cryohydrocytosis (a rare form
of stomatocytosis) associated with a cold-induced cation leak, hemolytic anemia, hepatosplenomegaly, cataracts, seizures, mental retardation, and movement disorder, previously reported by them, were associated with the SLC2A1 mutation Gly286Asp.

Given the rarity of such descriptions we can affirm that haploinsufficiency of GLUT1 is not always associate with hemolytic anemia; this outcome depends on the type of GLUT1 mutation and whether this leads to cation-leakage via erythrocyte membrane.
DIAGNOSIS

- **Lumbar Puncture**

The distinctive biomarker for GLUT1DS is low CSF glucose concentration, or hypoglycorrhachia (Rostein M et al., 2010).

Hypoglycorrhachia can also be found in other neurological conditions, such as prolonged seizures/status epilepticus, mitochondrial diseases, infectious meningitis, hypoglycemic states, subarachnoid haemorrhage, and meningeal carcinomatosis (Silver TS et al., 1976; Huang HR et al., 2006). If these conditions are ruled out, the presence of hypoglycorrhachia strongly indicates GLUT1DS.

Lumbar puncture should be performed in the fasting state, and the blood sample used for measurement of glucose concentration should be obtained immediately before the lumbar puncture to avoid stress-related hyperglycemia. The CSF-to-blood glucose ratio is superior to the absolute glucose level in CSF. Normal CSF-to-blood glucose ratios are above 0.6.

Initially, a CSF-to-blood glucose ratio of 0.33-0.37 (CSF concentration 40 mg/dl) (De Vivo DC et al., 1991) was set as the cut-off value for a diagnosis of GLUT1DS in suspected cases. However, with the increasing recognition of milder allelic variants, higher values are now being applied (see Table 1).

In our experience, milder phenotypes, especially ones characterized by movement disorders without epilepsy, can be associated with ratios of up to 0.59 (CSF glucose 60 mg/dl), even though, as affirmed by De Vivo (De Vivo DC et al., 2008) and Yang (Yang H et al. 2011) in the vast majority of cases (>90%), values are lower than 0.37.

These observations also indicate that the normal range for CSF glucose has never been defined properly. The risk that some patients with normal glycorrhachia might even go undiagnosed and untreated suggests that molecular analysis of the SLC2A1 gene should be used as an alternative gold standard for diagnosing GLUT1DS, when the condition is strongly suspected (Klepper J., 2012).

Several attempts have been made to correlate clinical severity with the degree of hypoglycorrhachia (Leen WG et al., 2010), but conclusive results are still lacking.

Reduced lactate concentration (below 1.4 mmol/L) (De Vivo DC et al., 1991; Ve Vivo DC et al., 2002) could be another CSF marker of GLUT1DS, although in our personal experience we have not found lactate levels to be significantly lower than normal (mean value 1.6 mmol/l). Klepper (Klepper J., 2012) recently affirmed that CSF lactate is never elevated in GLUT1DS.
<table>
<thead>
<tr>
<th>Year</th>
<th>Article</th>
<th>Tot. pt.</th>
<th>CSF mg/dl</th>
<th>Blood/CSF ratio</th>
<th>MR</th>
<th>Seizure</th>
<th>Motor signs</th>
<th>Other</th>
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<td>15</td>
<td>32</td>
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<td>Friedman et al.</td>
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<td>35</td>
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<td>31</td>
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<td>25</td>
<td>NA</td>
<td>+/– Ab, GTV, FS, MS</td>
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<td>PED, Cho, Dy</td>
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<td>3 26-31</td>
<td>0.33-0.38</td>
<td>+</td>
<td>Ab</td>
<td>PED, Dy, Dy, PS</td>
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<td>2 36</td>
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<td>Leen et al.</td>
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<td>Mullen et al.</td>
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<td>NA</td>
<td>-</td>
<td>Ab, GT, MS</td>
<td>PED</td>
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<td>Anheim et al.</td>
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<td>Byrne et al.</td>
<td>2 36</td>
<td>0.42 - .044</td>
<td>-</td>
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<td>PED, At, Cho</td>
<td>W</td>
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<td>Fuji et al.</td>
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<td>Fung et al.</td>
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<td>+</td>
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<td>Koy et al.</td>
<td>1 34</td>
<td>0.42</td>
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<td>0.42</td>
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<td>Yang et al.</td>
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<td>0.3</td>
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<td>Vieker et al.</td>
<td>1 34</td>
<td>0.39</td>
<td>-</td>
<td>MS, Ab, GTC</td>
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<td>-</td>
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<tr>
<td>2013</td>
<td>Tzadok et al.</td>
<td>8 30-49</td>
<td>0.39-0.54</td>
<td>+/-</td>
<td>GTC, MS, AA, Ab</td>
<td>At, PED</td>
<td>-</td>
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**Table 1**

- Erythrocyte 3-OMG uptake
As the GLUT1 gene is also expressed in erythrocytes, the finding of decreased uptake of 3-O-methylglucose (3-OMG) into erythrocytes could also serve to confirm a diagnosis of GLUT1DS. Pathogenic mutations cause haploinsufficiency, and therefore decrease 3-OMG uptake by approximately 50% (De Vivo DC et al., 1991). However, the phenotype associated with the T295M mutation in SLC2A1 shows normal 3-OMG uptake in erythrocytes, and thus leads to false-negative results in patients carrying pathogenic mutations (De Vivo DC et al., 2002). Furthermore, these investigations are not commercially available and are demanding in terms of protocol, time, sample size and sample quality (Klepper J, 2012).

- Molecular Analysis
Approximately 70-80% of patients carry SLC2A1 mutations (Pérez-Dueñas B et al., 2009). Genetic testing is commercially available worldwide. It should include PCR sequencing of all 10 exons, splice site, and the promotor region (Schneider SA et al., 2009; Pérez-Dueñas B et al., 2009). If it is negative, deletions/duplications within the SCL2A1 gene can be detected by multiplex ligation-dependent probe amplification (MLPA) (Leen WG et al., 2010). In SCL2A1-negative patients, GLUT1DS can be diagnosed only in the presence of clear hypoglycorrhachia. In such cases it is, indeed, reasonable to suspect GLUT1DS and to initiate KD treatment. An immediate response to the diet will support the diagnosis (Klepper J., 2012). These patients constitute a particularly interesting subset as they might carry defects in GLUT1 assembly, three-dimensional GLUT1 folding, GLUT1 trafficking to the cell, or GLUT1 activation (Klepper J & Leiendecker, 2007).
EEG FINDINGS

No characteristic EEG pattern in GLUT1DS has yet been identified, and it is possible to find a normal interictal EEG (Brockmann K, 2009; Pong AW et al., 2012).

Infants with GLUT1DS can present with multifocal spike discharges on EEG, underlying infantile focal seizures (non-generalized) that may include eye movements, cyanotic spells, etc. Subsequently, as the brain matures, these seizures become synchronized and clinically manifest themselves as generalized events associated with 3-4 Hz spike-and-wave discharges.

Tzadok (Tzadok M et al. 2013) maintain that the presence of polyspike wave discharges in the context of absence epilepsy without myoclonus or prominent aggravation of generalized polyspike wave discharges during sleep – which they did not usually observe in IGE patients – could be a characteristic sign. However, on the basis of our personal experience, we suggest that the above-mentioned features can also be present in “simple” forms of IGE. In our sample of GLUT1DS patients (article in press), we found that many “unusual” signs can be detected in idiopathic forms, such as the presence of atypical spike-and-wave discharges. In our view, these findings cannot be taken as characteristic signs.

On the contrary, some authors have reported improvement in the EEG findings post-prandially or after intravenous administration of glucose (Von Moers A et al., 2002). These EEG recordings could offer a simple screening test for GLUT1DS, even though, on their own, they cannot be considered diagnostic.

Martin-Valencia (Martin-Valencia I et al. in 2012) recently demonstrated in a mouse model of GLUT1DS that thalamocortical hypersynchronization is an important mechanism in GLUT1DS epileptogenesis due to impaired glucose uptake. Intrinsic cortical hyperexcitability arises from failure of internal thalamic inhibition, which causes propagation of excitation to the cortex; this could explain the frequent clinical presentation of absence epilepsy in patients with GLUT1DS. Moreover, because brain cells derive most of their energy from glucose, GLUT1 deficiency impairs also the synthesis of key molecules involved in energy production and neurotransmission, such as acetyl-CoA, tricarboxylic acid cycle intermediates and other derivatives.
Neuroimaging findings in patients with GLUT1DS are not significant. MRI scans of patients with GLUT1DS mostly show either normal findings (Brockmann K, 2009) or occasionally mild enlargement of inner and outer CSF spaces (Wang D et al., 2006; Klepper J & Leindecker B, 2007). Cerebral fluorodeoxyglucose PET in 14 patients with the classical GLUT1DS phenotype revealed a global decrease in glucose uptake in the cortex, more severe in the mesial temporal regions and thalami, and less marked in the basal ganglia (Suls A et al., 2008). The distinctive PET signature appears in early infancy and persists into adulthood regardless of disease severity or KD therapy. Although PET is readily available in clinical practice, the sensitivity and specificity of PET in diagnosis of GLUT1DS have not yet been established (Pascual JM et al., 2002).
TREATMENTS

- Ketogenic Diet

In the fasting state, brain glycogen storage is exhausted within minutes. The brain cannot utilize amino acids and fat to produce energy and, in the absence of glycogen, switches to ketones as an alternative fuel to maintain function. Ketones are generated in the liver from fatty acid degradation and enter the brain via facilitated diffusion mediated by the MCT1 transporter. This mechanism is particularly effective in infants and young children in whom ketone extraction and utilization is three-to-four-fold higher than in adults (Cremer JE et al., 1982).

The KD is a high-fat, carbohydrate-restricted diet that mimics the metabolic state of fasting. It has been used safely and effectively for decades in intractable childhood epilepsy (Klepper J, 2008). The classic KD, as developed by the Johns Hopkins University, uses long-chain triglycerides and consists of 4 g of fat to every 1 g of carbohydrate and protein combined (4:1 ratio) with supplemental vitamins and minerals. The spectrum of the KD has now increased considerably (Kossoff EH et al., 2009) and includes several alternative diets such as the classic KD with a 3:1 ratio, medium-chain triglycerides (MCTs), the modified Atkins diet (MAD) (which provides 10 g carbohydrates/day in children and 15 g/day in adults), and the low-glycemic index treatment (LGIT), which restricts the consumption of certain types of carbohydrate-containing foods.

Novel indications for KD include disorders of brain energy metabolism such as Pyruvate Dehydrogenase Deficiency (PDHP) and GLUT1DS (Veggiotti P et al., 2011).

Although, in epilepsy, the mechanism of action underlying the effectiveness of the KD is not yet clear (Kossoff EH & Hartman AL, 2012), in GLUT1DS it essentially provides an alternative fuel source. The effectiveness of the KD in GLUT1DS might be enhanced by its anticonvulsant action. The vast majority of GLUT1DS patients obtain efficient seizure control with the classical 4:1 or 3:1 formula, allowing anticonvulsant therapy to be withdrawn (Klepper J & Leidecker B., 2007). There are a few reports in which the KD was not fully effective and anticonvulsants were not completely eliminated (Freeman JM et al., 2007; Coman DJ et al., 2006).

The KD also has a positive effect on movement disorders such as hypotonia, ataxia, dystonia, and PED (Friedman JR et al., 2006; Gramer G et al., 2012).

The impact of KD treatment on developmental delay appears less prominent (Brockmann K, 2009).
However, several authors have reported a marked increase in alertness and activity in patients on the KD. The literature contains numerous reports of improved psychomotor impairment, but these are hard to verify in case-control studies. Our own experience has shown that introduction of the KD in the first years of life in patients with GLUT1DS guarantees a better cognitive outcome.

As the developing brain in the young child requires more energy, the KD should be started as early as possible whenever GLUT1DS is suspected.

There are also reports of voluntary intake of extensive amounts of fat-containing food in patients subsequently diagnosed as GLUT1DS (Ito S et al., 2008).

It remains unclear whether a rigorous KD is essential in GLUT1DS. Seizure and movement disorder control can be achieved by a 2:1 or 3:1 ketogenic ratio but the relationship between ketosis and neurodevelopmental outcome remains undetermined. MAD is also well tolerated and provides effective symptom control; furthermore, this diet has the advantage of being easy to prepare and more palatable (Stewart WA. Et al., 2001), which are important requirements for good compliance. On the contrary, there are no data about LGIT in GLUT1DS and studies are needed to investigate the possible effectiveness of this treatment. Finally, we have little direct experience of MCT therapy and therefore cannot express an opinion on this topic. We feel that MCTs could be integrated into the above diets to make them more manageable, however it is not our practice to use only MCTs in our drug-resistant epilepsy or GLUT1DS patients.

Figure 5 details, in addition to the diagnostic procedure, our clinical practice as regards the use of the KD in GLUT1DS patients at different ages and in different classes of severity.

There remain various challenges to be addressed. These include determination of the most efficient ratio and composition of the diets, and the development of treatment strategies in GLUT1DS adults.

The KD approach has advanced considerably over the past years, to the point that it is now considered a first-line therapy in infantile spasms, MAE (Doose syndrome), Dravet syndrome, and status epilepticus (including FIRES syndrome). KDs are also now being increasingly studied for neurological conditions other than epilepsy, including Alzheimer’s disease and cancer (Kossoff EH & Hartman AL, 2012). Further research could shed light on the mechanisms that may make metabolism-based therapy particularly helpful in terms of anticonvulsant and possibly neuroprotective effects.
Figure 4
Ictal EEG of an absence seizure in patient, showing generalized spike and wave (GSW) discharges (A). Interictally, disorganized background activity was apparent (B). After starting the ketogenic diet, background readily normalized (C). (Urbizu A et al., 2010)
Additional Measures

Treatment with a KD should be complemented by additional measures: pharmacological agents known to impair GLUT1 function, e.g. caffeine, phenobarbital, diazepam, chloral hydrate and tricyclic antidepressants, should be avoided (Wang D et al., 2006).

There are also reports of the use of alternative compounds for the treatment of GLUT1DS.

Alpha lipoic acid is an antioxidant that serves as a co-enzyme in energy metabolism. It neutralizes free radicals, improves cellular glucose uptake by stimulating the insulin signal cascade, reduces inflammation, and binds with metals. Alpha lipoic acid supplementation has been recommended in GLUT1DS on the basis of the observation that it improves glucose transport in cultured muscle cells via mobilization of the GLUT4 transporter from intracellular pools (De Vivo DC et al., 2002; Pong AW et al., 2012); however, to date there are no published data in humans to demonstrate its effectiveness.

Acetazolamide was reported to be beneficial for paroxysmal dyskinesias in a single patient with GLUT1DS (Koy A et al., 2011).

Triheptanoin is a triglyceride that has been used as an anaplerotic substrate in humans to treat inherited metabolic diseases such as pyruvate carboxylase deficiency and carnitine palmitoyltransferase II deficiency (Klepper J et al., 1999). It can produce ketone bodies with five carbon atoms that easily cross the blood-brain barrier and may enhance the effect of the regular ketone bodies as an alternative fuel for the brain. Although promising in theory, there is currently no clinical data to support the use of this compound in GLUT1DS.
FOLLOW-UP AND OUTCOME

Regular follow-up visits are needed after a diagnosis of GLUT1DS in order to monitor the patient’s clinical evolution. Particular attention should be paid to patients who begin KD treatment.

In accordance with the 2011 Italian consensus statement on the KD (Veggiotti et al., 2011), we recommend that follow-up of GLUT1DS children/adults include regular dietary and neurological evaluations and EEG, performed at least five times in the first year of treatment (after 1, 3, 6, 9, and 12 months). Annual cognitive and developmental assessments are also recommended, as are regular blood and metabolic evaluations.

In contrast to intractable childhood epilepsy patients, individuals affected by GLUT1DS should continue the diet into adolescence to meet the increased energy demands of the developing brain (Veggiotti P et al., 2010).

In our opinion, KD treatment can be useful in adulthood, too (paper in press), because even though epilepsy seems to disappear in adulthood, the movement disorder can persist unchanged and there are, at yet, no reliable data confirming a stable cognitive outcome in adult patients with GLUT1DS. More important, the KD might exert neuroprotective effects.

Low glucose concentrations in the CSF can lead to oxidative DNA damage and lipid peroxidation. Chronic ketosis limits the generation of reactive oxygen species and boosts energy reserve capacity, which is important in sustaining the electrophysiological activities essential for performing brain function (Anheim M et al., 2011).

Furthermore, in GLUT1DS patients, KD therapy could be beneficial to organs expressing GLUT1 at high levels such as the retina, colon, ovaries and testicles (Klepper J, 2008). For these reasons, if there are no serious side effects, we recommend lifelong KD treatment in GLUT1DS patients, even if symptom control is not complete. The defect caused by the failure of glucose to cross the blood-brain barrier must be bypassed in order to reduce the long-term risks associated with the disease.

In our experience, compliance is much better in GLUT1DS than in the other conditions for which KD treatment is indicated. According to the literature there is a good level of satisfaction: up to 75% patients consider the diet effective and 50% tolerate and accept it (Coman DJ et al., 2006).

The question of when to discontinue AEDs in patients with GLUT1DS is not really debated in the literature. According to our protocol, if seizures and non-epileptic paroxysmal events have disappeared, gradual
reduction of AEDs can start from the first year after the introduction of the KD. If the diet does not completely control the symptoms, AEDs are continued at the most effective and best tolerated dose. No information is available on the long-term efficacy and tolerability of the treatment in GLUT1DS patients, but non-GLUT1DS seizure-free epileptic patients on the diet for >10 years have been reported (Groesbeck DK et al., 2006).
GLUT1 deficiency syndrome results from impaired glucose transport into the brain. Recently the clinical spectrum of GLUT1DS has been broadened and it is becoming less meaningful to consider the disease in terms of “classical” and “non-classical” phenotypes.

On the basis of current knowledge, this disease should be suspected (figure 5) and a CSF investigation (with determination of the CSF-to-blood glucose ratio) is strongly recommended in:
- any seizures that are refractory and/or influenced by fasting, especially if associated with mild neurological (pyramidal or extra-pyramidal) signs and/or mental impairment and/or atypical EEG features and/or a family history of epilepsy or movement disorder.
- any history of unexplained paroxysmal events — especially if PED — associated with previous self-limited seizures and/or mental impairment and/or a family history of epilepsy or movement disorder.

It is essential diagnose this entity as early as possible to allow prompt compensation, through the KD, for the brain’s lack of fuel. Early identification of children with GLUT1DS is important in order to avoid submitting them to possibly ineffective or potentially detrimental treatment with anticonvulsants, and to ensure that their brains are provided with an alternative energy source during a time of increased cerebral metabolism.

On the basis of current information, it is not possible to say with absolute certainty that the condition of GLUT1DS patients never deteriorate. The hallmark deficiency of glucose in the CNS, if allowed to persist for many years, could be responsible for brain atrophy, which could be observed on the MRI scans of these patients, and for a moderate but steady reduction of IQ, as well as for the maintenance of more or less drug-resistant epilepsy and a disabling movement disorder. The few GLUT1DS adult patients identified to date do not provide sufficient evidence to clarify our doubts in this regard.

On-going research to discover pathogenic mechanisms underlying different phenotypes, the availability of new animal models of GLUT1DS, the increase in patient numbers, and the diffusion of knowledge of this relatively “new” disease could help us to provide answers to some of the many still open questions on GLUT1DS.
**Lumbar Puncture** in the morning before breakfast, blood sample before lumbar puncture

**Eventual intravenous infusion of glucose during EEG recording**

**Acquired microcephaly**
**Developmental delay**
**Pharmacoresistant epilepsy**
**Complex movement disorder**

**Pre/post prandial EEG**

**MOLECULAR ANALYSIS**
**PCR sequency**
**MLPA (always!)**

**KETOGENIC DIET**

- **4:1**
  - **In**
  - **Ps**
  - **Sc**
  - **Ad**
  - **At**

- **3:1**
  - **In**
  - **Ps**
  - **Sc**
  - **Ad**
  - **At**

- **MAD**
  - **Sc**
  - **Ad**
  - **At**

**EPILEPSY**

- **Familial IGE**
- **Absence**
- **Myoclonic astatic Epilepsy nos**
  (with fasting exacerbating seizures)

**PAROXYSMAL EVENTS**

- **Fluctuating during the day and exacerbated by environmental stressors**
- **PED**
- **Ataxia**
- **Choreoathetosis**
- **Action limb dystonia**
- **Migraine**
- **Weakness**

**Severe phenotype**

**Mild phenotype**

**LOW**

+- various degrees of developmental delay

**Figure 5**

*Diagnostic and therapeutic GLUT1 DS flow chart*
AIM OF THE STUDY

GLUT1 deficiency syndrome (GLUT1DS, OMIM 606777) is a treatable neurological disorder caused by a deficiency of glucose transporter type 1 (GLUT1) at the blood-brain barrier and in brain cells which results in impaired glucose transport into the brain. The clinical manifestations of GLUT1DS include developmental delay, movement disorders and acquired microcephaly.

GLUT1DS was first described in 1991 by De Vivo (De Vivo et al. 1991) and seven years later a molecular basis for the defect in GLUT1-mediated glucose transport was confirmed (Seidner et al. 1998). Since 1991 few patients have been identified in southern and eastern European countries and only two papers (Zorzi et al. 2008; Verrotti et al., 2012) on the diagnosis and treatment of GLUT1 deficiency have been published in Italy. It is therefore likely that GLUT1DS is currently underdiagnosed. This situation needs to be addressed as prompt diagnosis would allow early introduction of the ketogenic diet (KD) that could reduce the frequency of seizures and the severity of the movement disorders, and improve patients’ behaviour and alertness (Klepper 2008). Glucose trasporter-1 deficiency syndrome is caused by mutations in the SLC2A1 gene (OMIM 138140) which maps to the short arm of chromosome 1 (1p35-31.3) (De Vivo et al. 2002) This is the only gene known to be associated with GLUT1DS. In familial cases the condition is inherited as an autosomal dominant trait with complete penetrance, although most detected SCL2A1 mutations are de novo (Klepper et al., 2003).

Here, we present data on a group of 16 genetically confirmed GLUT1DS Italian patients, not previously reported, comparing their clinicogenetic characteristics with those of five genetically negative patients. Finally, we discuss the variability of the clinical phenotypes and highlight the characteristics of this recognizable syndrome.
MATERIALS AND METHODS

-Patients

Over an eight-year period (2006-2013), 21 patients meeting the criteria for suspected GLUT1DS (Pearson et al., 2013), in particular early-onset or atypical epilepsy, developmental delay and movement disorders (Figure 5), underwent lumbar puncture at our reference centers. All were of Italian origin and born to non-consanguineous parents. The clinical, biochemical and genetic features of patients #1, #2 and #3 are already described in previous papers by our group (Zorzi et al., 2008; Veggiotti et al., 2009).

In 15/21 patients, lumbar puncture was performed in the fasting state (after 5-6 hours of fasting), and the blood sample for glucose measurement was obtained immediately before the procedure to avoid stress-related hyperglycemia (Wang et al. 2005).

Normal CSF-to-blood glucose ratios are above 0.6. Hypoglycorrhachia occurs in GLUT1DS but can also be found in other neurological conditions such as prolonged seizures/status epilepticus, mitochondrial diseases, infectious meningitis, hypoglycemic states, subarachnoid haemorrhage and meningeal carcinomatosis (Huang et al. 2006). After excluding these conditions, the presence of a CSF-to-blood glucose ratio of under 0.6 was considered suspicious for GLUT1DS and genetic analysis was performed (Klepper & Leendecker, 2007).

In remaining 6 patients also presenting clinical signs of the disease were submitted to SLC2A1 mutation analysis even though lumbar puncture was not performed for different reasons: patients #12 and #14 were obese, the family of patient #11 refused, while patients #9, #13 and #15 were the parents of probands. All the blood samples blood samples were collected after obtaining written informed consent, from patients and/or the parents. The investigations fulfilled our institution’s ethical rules for human studies.
<table>
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Table 1 - Clinical characteristics of GLUT1DS patients

Abbreviations: F, female; M, male; CSF, cerebrospinal fluid; NA, not available; Y, yes; N, no; SFS, focal simplex seizure; CFS, complex focal seizure; ABS, absence seizure; GTC, generalised tonic-clonic seizure; MS, myoclonic seizures; MAs, myoclonic astatic seizures; MD, movement disorder; C, continuous; PED, paroxismal exertion-induced dyskinesia; PND, paroxysmal non-exertion-induced dyskinesia, ataxia; d, dystonia; c, choreoatetosis; t, tremor; KD, ketogenic diet; M, migraine; DS, dysarthric speech; Mi, Prognatysm with dental malocclusion and/or supernumerary teeth; W, weakness, M, myoclonias.

*Grade: - >50°p (normal); +/- 25°-50°p; + <25°p.

*Grade: - none (IQ 80-100); +/- borderline (IQ 70-80); + mild (IQ 50-70); ++ moderate (IQ 35-50); severe (IQ 20-35)

*Grade: +, epilepsy or movement disorder alone; ++, epilepsy + movement disorder; +*, movement disorder and IQ amelioration.
Table 3 - Clinical characteristics of patients with hypoglycorrachia SLC2A1 negatives

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<th>Patient</th>
<th>Gender</th>
<th>Age at study (y)</th>
<th>CSF/blood glucose ratio</th>
<th>Microcephaly</th>
<th>Spasticity</th>
<th>Seizures presence</th>
<th>Seizures onset (months)</th>
<th>Seizure type</th>
<th>MD presence</th>
<th>MD type</th>
<th>MD subtype</th>
<th>MD onset (months)</th>
<th>Other</th>
<th>KD response</th>
</tr>
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<tbody>
<tr>
<td>17</td>
<td>F</td>
<td>13</td>
<td>0.68</td>
<td>-</td>
<td>++</td>
<td>n</td>
<td>y</td>
<td>48</td>
<td>ABS GTC SFS</td>
<td>y</td>
<td>C</td>
<td>at</td>
<td>12</td>
<td>Ds Mi Bd</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>6</td>
<td>0.52</td>
<td>-</td>
<td>++</td>
<td>n</td>
<td>n</td>
<td>/</td>
<td>/</td>
<td>y</td>
<td>C</td>
<td>a</td>
<td>12</td>
<td>Ds W</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>5</td>
<td>0.50</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>n</td>
<td>3</td>
<td>ABS GTC SFS</td>
<td>y</td>
<td>C</td>
<td>a c d</td>
<td>12</td>
<td>Mi ++</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>3</td>
<td>0.51</td>
<td>-</td>
<td>+/-</td>
<td>n</td>
<td>y</td>
<td>?</td>
<td>MAS MS</td>
<td>n</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>NA</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>5</td>
<td>0.61</td>
<td>+</td>
<td>+/-</td>
<td>n</td>
<td>y</td>
<td>ABS GTS</td>
<td>y</td>
<td>PND</td>
<td>c d</td>
<td>5</td>
<td>Bd</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; CSF, cerebrospinal fluid; NA, not available; Y, yes; N, no; SFS, focal simplex seizure; ABS, absence seizure; GTC, generalised tonic-clonic seizure; MS, myoclonic seizures; MAS, myoclonic atatic seizures; MD, movement disorder; C, continuous; PND, paroxysmal non-exertion induced dyskinesia, ataxia; d, dystonia; c, choreoatetosis; t, tremor; KD, ketogenic diet; Ds, dysarthric speech, Mi, Prognatism with dental malocclusion and/or supernumerary teeth; Bd, behavior disorder, W, weakness.

*Grade: - >50°p (normal); +/- 25°-50°p; + <25°p.

*Grade: - none (IQ 80-100); +/- borderline (IQ 70-80); + mild (IQ 50-70); ++ moderate (IQ 35-50); severe (IQ 20-35)

*Grade: +, epilepsy or movement disorder alone; ++, epilepsy + movement disorder; +*, movement disorder and IQ amelioration.
Mutation analysis of SLC2A1

The study sample comprised 15 females and 6 males, aged 3-57 years, with various degrees and combinations of epilepsy, movement disorders and mental retardation. The clinical signs and laboratory data for the study patients are presented in Tables 1 and 2.

Genomic DNA was extracted from peripheral blood mononuclear cells. Coding exons of the Solute Carrier family 2 (facilitated glucose transporter) member 1 gene (SLC2A1) were amplified from genomic DNA (~200 ng) by polymerase chain reaction (PCR). After the PCR purification the amplicons were processed for sequence analysis. Thereafter, they were purified and finally screened for sequence variations by forward and reverse direct sequencing.

Pathogenic mutations were found in 16 patients (Table4).

All the identified sequence variations were confirmed by sequencing a forward and reverse independent PCR product. The sequencing results were compared to a reference sequence (NCBI). The NCBI website was checked for the presence of any missense, synonymous and intronic variants identified.

The effect of new SLC2A1 mutations on protein structure or function was analyzed using three prediction programs: PolyPhen (http://genetics.bwh.harvard.edu/pph/) SNAP (http://cubic.bioc.columbia.edu/services/SNAP/submit.html), and PMUT (http://mmb2.pcb.ub.es:8080/PMut). The likelihood of the mutation having a functional impact on the protein, based on the alignment of evolutionarily related proteins, was calculated using the pathogenicity predictor Panther software.

Multiplex ligation-dependent probe amplification (MLPA) analysis

The patients with negative sequence analysis of the SLC2A1 gene were investigated with the MLPA method to exclude the presence of exonic deletions or duplications. 5 μL of genomic DNA (from 50 to 100 ng/μL) was incubated with SALSA MLPA Kit P138 SLC2A1 (MRC-Holland) in accordance with the manufacturer's specifications. The MLPA data were analyzed with the Coffalyser Program (MRC-Holland) but none of the resulting five sequencing-negative patients (5/21) was found to be positive for SLC2A1 exonic deletions or duplications.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Mutation</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Type of mutation</th>
<th>Family member analysis</th>
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<tr>
<td>1</td>
<td>W48X</td>
<td>na</td>
<td>na</td>
<td>nonsense</td>
<td>Father (-) Mother (-) Sister (-)</td>
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<tr>
<td>2</td>
<td>Q283X</td>
<td>na</td>
<td>na</td>
<td>nonsense</td>
<td>Father (-) Mother (-) Sister (-)</td>
</tr>
<tr>
<td>3</td>
<td>R126C</td>
<td>Exon 4</td>
<td>c.376C&gt;T</td>
<td>missense</td>
<td>Father (-) Mother (-)</td>
</tr>
<tr>
<td>4</td>
<td>C107P</td>
<td>na</td>
<td>na</td>
<td>missense</td>
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<tr>
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<td>Exon 5</td>
<td>c.667C&gt;T</td>
<td>missense</td>
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</tr>
<tr>
<td>6</td>
<td>R153C</td>
<td>Exon-Intron 9</td>
<td>na</td>
<td>missense</td>
<td>NA (adopted child)</td>
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<td>c.1372C&gt;T</td>
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</tr>
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<td>8</td>
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<td>Exon 10</td>
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<td>missense</td>
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<td>c.376C&gt;T</td>
<td>missense</td>
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<tr>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>c.1475delG_+IdelG</td>
<td>nonsense</td>
<td>Father (-) Mother (-)</td>
</tr>
<tr>
<td>11</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Father (-) Mother (-)</td>
</tr>
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<td>12</td>
<td>R153C</td>
<td>Exon 4</td>
<td>c.457C&gt;T</td>
<td>missense</td>
<td>Father (-) Mother (+)</td>
</tr>
<tr>
<td>13</td>
<td>R153C</td>
<td>Exon 4</td>
<td>c.457C&gt;T</td>
<td>missense</td>
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<tr>
<td>14</td>
<td>G91A</td>
<td>Exon 3</td>
<td>c.797 G&gt;C</td>
<td>missense</td>
<td>Father (+) Mother (-)</td>
</tr>
<tr>
<td>15</td>
<td>G91A</td>
<td>Exon 3</td>
<td>c.797 G&gt;C</td>
<td>missense</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>R92W</td>
<td>Exon 3</td>
<td>c.274C&gt;T</td>
<td>missense</td>
<td>Father (-) Mother (-)</td>
</tr>
</tbody>
</table>

*Table 4 - pathogenetics mutation in our 16 SLC2A1 patients*
RESULTS IN SLC2A1-POSITIVE PATIENTS

- Clinical characteristics

Glucose transporter-1 deficiency syndrome was genetically confirmed in 16 patients, 11 females and 5 males, with an age range of 5-57 years at the time of their diagnosis and enrollment in the study. The clinical signs and laboratory data for the positive patients are detailed in Table 2.

In all these patients, pregnancy, delivery and the neonatal period had been uneventful. Five patients (5/16) had microcephaly (head circumference below or equal to the 25th percentile for age). Various degrees of mental retardation were present in 12 patients.

In 14 patients (mean age 31.8), epilepsy was an important sign, and in all but one (13/14) it was the first symptom recognized. Four of these patients presented with early-onset epilepsy (5-8 months) and nine had, at some time during their lifetime (more frequently in infancy), shown drug resistance.

Seizure types varied and included absence seizures (11/16), usually atypical absence or drug-resistant ones, focal complex (5/16), generalized tonic-clonic (4/16), myoclonic-astatic (1/16), myoclonic (1/16), and focal simple (1/16) seizures usually reported to be more frequent in infancy.

![Seizures type](image)

Figure 6
Different seizures type in SLC2A1 positive patients. MS, Myoclonic seizures; MAS, Myoclonic astatic seizures; SFS, Simple focal seizures; GTC, Generalised tonic-clonic seizures, CFS, complex focal seizures; ABS, absences.
All but two of the patients showed movement disorders: paroxysmal exercise-/stress-/fasting-induced (14/16) and/or continuous (7/16). Movement disorders in most cases began in childhood/adolescence (at ages ranging from 8 to 240 months, mean 68.3 months) and usually after seizure onset; only one patient (#5) had a continuous movement disorder (ataxia) prior to seizure onset.

Other associated clinical signs included spasticity (5/16), dysarthria (6/16), weakness on awakening or in the fasting state (6/16), migraine (3/16) and a characteristic gestalt sign: prognathism (4/16).
- **Biochemistry and genetics**

Laboratory examination revealed various degrees of hypoglycorrhachia in all the SLC2A1-positive patients who underwent lumbar puncture (CSF/blood glucose ratio: 0.33-0.51, mean 0.36) but no correlation emerged between CSF glucose levels and clinical severity, in agreement with the findings of other authors (15) Mullen et al. 2010, (16) Tzadok et al., 2013).

Measurement of 3-OMG uptake by erythrocytes was not performed.

Table 4 summarizes the results of the SLC2A1 gene analysis.

Direct sequencing of SLC2A1 revealed 13/16 missense mutations and 2/16 nonsense mutations.

In all the genetically positive patients direct sequencing of the gene revealed de novo mutations and it was not therefore necessary to perform MLPA.

- **EEG and imaging**

At the time of GLUT1DS diagnosis, interictal EEG (Van Moers et al., 2002) tracings showed epileptiform discharges in 9/16 patients usually with diffuse background slowing and a generalized 2-3.5 Hz spike-wave pattern. It is important to note that at the time of diagnosis and enrollment, all the adult patients had been seizure-free for several years, whereas only two of the children (patients #6 and #7) had epilepsy.

Routine laboratory investigations and full metabolic work-up were unremarkable. No abnormalities were detected on electrophysiological studies, including motor and sensory nerve conduction velocities, and visual and auditory evoked potentials. In most patients, MRI showed normal findings, with the exception of the presence of moderate generalized cortical atrophy in patient #3, and moderate atrophy of the cerebellar vermis in patient #2, all findings that the neuroradiologists did not consider significant or attributable to GLUT11DS.
RESULTS IN SLC2A1-NEGATIVE PATIENTS

- Clinical characteristics

In five patients (3 females and 2 males, aged 3-13 years at study entry), genetic analysis (with gene sequencing and MLPA) failed to confirm the diagnostic suspicion. The clinical signs and laboratory data for the genetically negative GLUT1DS patients are listed in Table 3.

In all these patients, pregnancy, delivery and the neonatal period had been uneventful. Three patients (3/5) had microcephaly (head circumference below or equal to the 25th percentile for age). Various degrees of mental retardation were present in all five patients.

In three patients epilepsy was an important clinical feature, even though continuous movement disorder and/or developmental delay were the first signs recognized. With regard to epilepsy, it is noted that only one patient presented with early-onset seizures (3 months).

Seizure types varied and included absence seizures (3/5), usually atypical absence or drug-resistant ones, generalized tonic-clonic (3/5), myoclonic-astatic (1/5), myoclonic (2/5), and focal simple (1/5) seizures.

![Seizures type](image)

**Figure 9**

Different seizures type in SLC2A1 negative patients. ABS, absences; GTC, Generalised tonic-clonic seizures; SFS, Simple focal seizures; MS, Myoclonic seizures; MAS, Myoclonic astatic seizures.
Movement disorders were present in all but one of the genetically negative patients: paroxysmal non-exercise-induced in two patients (2/5) and subtle, permanent types in three (3/5): mainly ataxia (3/5), but also dystonia (2/5), choreoathetosis (1/5) and tremor (1/5), alone or in various combinations. None of these patients presented paroxysmal exercise-/stress-/fasting-induced movement disorders.

The onset of the movement disorder occurred very early in the SLC2A1-negative compared with the SLC2A1-positive patients (at an age of 5 to 12 months, mean 10.5 months) and in close temporal proximity to the seizure onset.

Other associated clinical signs included spasticity (1/5), dysarthria (2/5), behavioural disturbance (2/5) weakness on awakening or in the fasting state (1/5), and prognathism (2/5).
- Biochemistry and genetics

Lumbar puncture revealed various degrees of glycorrhachia in all the SLC2A1-negative patients (CSF-to-blood glucose ratio: 0.50–0.68, mean 0.59).

Although the glycorrhachia level (and therefore also the CSF-to-blood glucose ratio) was quite high in these subjects, genetic investigation was nevertheless performed on the strength of their clinical picture, which, in line with literature data (Wang et al. 2005; Leen et al., 2010.), was highly suggestive of GLUT1DS. According to recent reports (Mullen et al., 2012; Tzadok et al., 2013), GLUT1DS patients can have higher levels of CSF glucose (up to 49 mg/dl) and higher CSF-to-blood glucose ratios (up to 0.54), albeit without the latter ever reaching 0.60.

- EEG and imaging

In the SLC2A1-negative patients, interictal EEG tracings at diagnosis showed, in comparison with those of the SLC2A1-positive patients, a wider range of epileptiform abnormalities and discharges: all showed poor organization of activity associated with diffuse background slowing. Multifocal atypical spike-and-wave abnormalities without any clear localization were observed, while in myoclonic/myoclonic-astatic EEG patterns polyspikes during weakness, sometimes associated with myoclonias, were registered. In one patient (#17), pre- (fasting) and postprandial EEG recordings documented a remarkable improvement in the EEG after food intake.

Routine laboratory investigations and full metabolic work-up – as above – were unremarkable. No abnormalities were detected on neuroimaging or electrophysiological studies including motor and sensory nerve conduction velocities, and visual and auditory evoked potentials.
RESPONSE TO THE KETOGENIC DIET

Eight (8/16, 50%) of the SLC2A1-positive patients and one of the SLC2A1-negative patients began KD treatment (table 4).

Patients were initially started on the classical 3:1 ketogenic ratio (g lipids: g glucose) but in some selected and strictly monitored cases we were able to reduce the ratio, in one case to 1.8:1, maintaining the same therapeutic efficacy with stable beta-hydroxybutyrate levels of around 2 Mmol/l.

The patient with negative genetic findings (#22) in whom the KD (4:1) was prescribed as treatment for multidrug-resistant epilepsy and encephalopathy deserves a special mention. This patient obtained a marked seizure reduction (to disappearance), and a remarkable improvement in the EEG (see Figure 2). Paroxysmal exertion-induced dystonia (PED), a complex movement disorder, disappeared in all the SLC2A1-positive patients after they started the KD, a dramatic result that was not replicated for continuous movement disorders like ataxia or chorea. Indeed, only three patients (#1, #2, #3) showed a mild improvement (see table 4) in these disorders; at present we have no evidence to suggest that these symptoms might be significantly improved by starting the diet earlier. What we can affirm is that the intermitting worsening associated with exercise or fasting disappeared in all the patients on the KD. We also observed a gradual normalization of the EEG in all the patients and disappearance of seizures in the two (#7 #8) who presented clinical seizures. As a result, all the patients were able to come off antiepileptic drugs (AEDs) after about the first year of treatment.

The clinical improvement was maintained over time, leading us to recommend permanent continuation of the KD therapy. Patient-perceived improvements in cognitive function, alertness and activity after starting the KD were reported in five (5/8) of the SLC2A1-positive patients receiving the treatment. It is also worth noting an IQ improvement (albeit not statistically significant) in patient #8, documented by an increase from 79 to 89 on the Wechsler Infant Intelligence Scale, after two years on the KD. The patients showed, in the long term, a high level of long-term compliance with this dietary regimen.

As Klepper underlines in his review (Klepper, 2012), available data on the long-term effectiveness and tolerability of KD in GLUT1DS are limited, especially in adult patients.

We have recently reported a six-year follow-up of three adults on KD, describing their clinical evolution, biochemical data, side effects and compliance (see next chapter). Our experience suggests that in order to eliminate movement disorders and seizures in these patients, and therefore improve their quality of
Table 5 - Follow up of GLUT-1 patients on ketogenic diet.

<table>
<thead>
<tr>
<th>Patient 1 CG</th>
<th>Patient 2 DP</th>
<th>Patient 3 RD</th>
<th>Patient 4 CA</th>
<th>Patient 5</th>
<th>Patient 7</th>
<th>Patient 8 MA</th>
<th>Patient 10 PC</th>
<th>Patient 19 RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>T1</td>
<td>T2</td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
</tr>
</tbody>
</table>
| Paroxysmal Dyskinesias | PED | - | - | PNKD | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | PED | - | - | P...
DISCUSSION

Glucose transporter-1 deficiency syndrome was originally described in 1991 as a developmental encephalopathy characterized by infantile-onset refractory epilepsy, cognitive impairment and mixed motor abnormalities, including spasticity, ataxia and dystonia. (De Vivo et al., 1991)

Since then, the spectrum of GLUT1DS has broadened and varying degrees of severity are now recognized. Neurological features may be divided into three cardinal domains: seizures, movement disorders and cognitive/behavioral disturbances (Klepper, 2012). While the classical GLUT1DS phenotype is characterized by persistent symptoms involving all three domains, patients with milder phenotypes may experience symptoms in only one or two domains, and symptoms may be either intermittent or persistent (Pearson et al. 2013).

We conducted SLC2A1 gene analysis in 21 Italian patients presenting with clinical features commonly associated with GLUT1DS: early-onset and/or drug-resistant epilepsy, a complex movement disorder, and some degree of cognitive impairment. Sixteen of these cases were found to be genetically positive for SLC2A1. This is the first report of a large number of Italian patients with GLUT1DS.

Seizures in our SLC2A1-positive series – as previously described (Mullen et al., 2011; Tzadok et al, 2013; Verrotti et al., 2012; Pong et al., 2012) – presented mainly in early infancy and were refractory to conventional AED treatment. Only one of the genetically negative patients reported an early onset (#19).

Various seizure types were observed, without significant differences emerging between the two groups. In the SLC2A1-positive patients the most common seizure type was absence (69 %), followed by partial (37.5%), generalized tonic-clonic (25%), and finally myoclonic and myoclonic-astatic (12.5%) seizures; 37.5% of patients had more than one seizure type. Our sample reflected what can be found in the literature (Arsov et al., 2012): simple or complex partial seizures appear mainly in infancy (Klepper, 2012.), whereas myoclonic and generalized seizures (Brockmann., 2009), -in particular absence epilepsy (Mullen et al., 2010), myoclonic-astatic epilepsy (Vieker et al., 2012; Gökben et al., 2011; Mullen et al., 2011), or juvenile myoclonic epilepsy (especially with atypical features)- tend to appear in childhood. In accordance with previous reports (Klepper, 2012.), Brockmann., 2009, Pearson et al. 2013), the EEG findings were not found to differ significantly between the groups.

It is interesting to note that a positive EEG response to food intake was found in only one SLC2A1-negative patient (#17) (von Moers et al., 2002; Brockmann et al., 1999).
Movement disorders differed markedly between the two groups: in the SLC2A1-positive patients, PED (87.5%), either dystonia (principally lower limb dystonia precipitated by sustained walking or running) or chorea, was the most frequent movement disorder, followed by permanent movement disorders (44%): ataxia or chorea. Paroxysmal non exertion-induced movement disorder was found in only two genetically positive patients (# 15 and #16). The paroxysmal episodes showed highly variable duration: from a few minutes to several hours.

These episodes first appeared at a mean age of 5.7 years, some years after seizure onset (mean age 2.65 years), while the average age of the patients at GLUT1DS diagnosis was 22 years, highlighting a considerable lag time to diagnosis.

In the genetically negative group we observed mainly subtle, permanent movement disorders (ataxia 60%, dystonia 40%, choreoathetosis 40%), but also paroxysmal non-exertion-induced ones (40%). None of the patients in this group presented PED. This finding, together with the findings in the positive patients, suggests that PED is the MD most characteristic of GLUT1DS (Weber et al., 2008; Suls et al., 2008; Schneider et al., 2009). Weakness and intermittent worsening of the movement disorder during exercise or fasting were also seen in both of the study groups (present in 37.5% of the positive and 20% of the negative patients). This is a pattern already described in GLUT1DS patients (Pérez-Dueñas et al., 2009; Koy et al., 2011).

Various degrees of mental impairment were seen in this Italian series of GLUT1DS. In the SLC2A1-positive group, 12.5% had severe mental retardation, 19% moderate mental retardation, while 50% recorded a lower or borderline IQ. Not all the patients in this series showed individual scores in the mentally retarded range: four patients (25%) had a normal IQ, albeit usually lower than 100. The genetically
negative group showed a higher rate of mental impairment: 20% severe, 40% moderate, 40% lower or borderline IQ. In this regard, our sample substantially reflected those described in the literature (Leen et al., 2010).

Dysarthria, reported to be a common sign (Pearson et al. 2013; Yto et al. 2011), was present in 37.5% of our positive patients, with halting speech, pauses, articulation errors and dropping of word endings occurring most commonly; however, dysarthria was also present in 40% of our negative patients, suggesting that in this small sample, it may not necessarily be linked to GLUT1DS.

Migraine was reported in only three members of the SLC2A1-positive group: two adults, from the same family (patient #12-#13), and one child (#4), but these patients did not share any other features. No migraine was reported in the negative group. These findings suggest that migraine cannot be considered a particularly characteristic sign of GLUT1DS.

Microcephaly was not a key feature in most of the patients: it was seen mainly in positive patients with a severe phenotype (#1, #2, #11, #16) and in two negative patients (#19 and #21).

Another extra-neurological sign observed was prognathism (present in 25% of the positive cases): while not essential for the diagnosis, this could be a gestalt sign worth remembering.

When GLUT1DS is clinically suspected, the most important laboratory feature for the diagnosis is lowered CSF glucose (hypoglycorrachia) (Leen et al., 2010; Pearson et al. 2013; Rotstein et al., 2010) in the absence of hypoglycemia: in the presence of this finding, the exclusion of various other conditions
characterized by reduced CSF glucose levels is strongly suggestive of GLUT1DS. Lumbar puncture should be performed in the fasting state, and the blood sample for glucose measurement should be obtained immediately before the lumbar puncture procedure.

In this study, we also performed genetic screening in patients with higher CSF-to-blood glucose ratios (i.e. up to 0.68) whose clinical features were strongly suggestive of GLUT1DS (see Tables 1 and 2). All the SLC2A1-positive patients who underwent lumbar puncture had CSF/blood glucose ratios in the range 0.33-0.51 (mean 0.36), while the higher ratios (from 0.61 to 0.68) were found in the SLC2A1-negative patients.

These findings reinforce our opinion that even though CSF analysis can be misleading in some cases, a ratio below 0.40 (without any other cause of hypoglycorrhachia) is highly suggestive of GLUT1DS, while a value of between 0.40 and 0.60 is less clear-cut and warrants genetic investigation. Instead, ratios of over 0.60 have not, to date, been described in association with GLUT1DS.

A diagnosis of GLUT1DS can be further supported by the results of GLUT1 Western blot analysis of erythrocyte membranes and measurement of glucose uptake into erythrocytes, performed in order to determine GLUT1 function, even though negative findings do not rule out the diagnosis (Klepper et al., 1999; E. Maratou et al., 2007). Ultimately, GLUT1DS should be confirmed or excluded by mutation analysis of the SLC2A1 gene.

Patients with GLUT1DS show substantial genotypic and phenotypic variability. An analysis of genotype-phenotype correlations was recently performed – for the first time in GLUT1DS – in 57 patients (Leen
et al., 2010). The authors clustered mutations into three types: type A (missense mutations), type B (nonsense, frame shift, splice site, translation initiation mutations), and type C (multiple exon deletions). Mild mental retardation was found to be correlated with type A mutations, whereas movement disorders were more frequently seen in patients with type B and C mutations.

We were not able to conduct such a correlation analysis because we found 12 missense mutations and only three nonsense mutations in our sample. Moreover, our series contained three families with autosomal dominant transmission of the disease (patients #8 and #9; patients #12 and #13; patients #14 and #15) who, as shown in Table 3, nevertheless displayed considerable phenotypic variability.

At present, it appears particularly interesting to focus on those patients who, despite having a clinical picture that is highly suggestive of GLUT1DS, a low glycorrhachia value and possibly a good response to the KD, are negative on SLC2A1 gene sequencing and MLPA. In these patients, sequencing of the entire exome could be warranted in order to search for possible other genes implicated in GLUT1 protein assembly, three-dimensional GLUT1 folding, GLUT1 trafficking to the cell, or GLUT1 activation.

With regard to therapeutic strategies, the most striking finding, observed in all our SLC2A1-positive patients, was the disappearance of the movement disorders, in particular PED (Veggiotti et al., 2009; Friedman et al. 2009), almost immediately after starting the KD treatment; moreover, they experienced no recurrence (Veggiotti et al., 2011). In all the patients with epilepsy, we observed complete disappearance of seizures and gradual normalization of EEG background activity with disappearance of epileptic discharges. In all cases, these changes allowed AEDs to be withdrawn after the first year of treatment.

Our experience with the classical 3:1 ratio, or lower, provides confirmation of the long-term effectiveness of a less restrictive KD, in terms of the proportions of fat to no-fat foods, which can result in levels of beta-hydroxybutyrate that are lower than those obtained with the most commonly used 4:1 ratio, but nevertheless sufficient to obtain adequate ketosis for symptom control (see next chapter).

In this setting, there is a need for further long-term follow-up studies and more studies addressing pathogenic mechanisms and potential new treatment strategies.
CONCLUSION

GLUT1DS is increasingly recognized as a great mimicker of various neurological conditions, and a high index of suspicion is therefore important in order to avoid false positives. In this context lumbar puncture, a simple investigation, might constitute the first diagnostic step; conversely, mutational analysis should be performed only in patients with highly suggestive clinical findings and low CSF glucose (<50 mg/gl or ratio <0.60).

Treatment with KD can provide effective control of seizures and paroxysmal events, and help to improve weakness and continuous movement disorders.

It is worth underlining that the KD treatment led to seizure disappearance and a remarkable improvement in the EEG findings in the one patient who was found to have negative genetic findings (#22) but hypoglycorrhachia (0.45 mg/dl, ratio 0.50), severe mental retardation, complex movement disorder and multidrug-resistant epileptic encephalopathy (Table 4, Figure 2).

We believe that sequencing of the entire exome in a small number of patients with a similar phenotype could lead to the detection of other genes implicated in GLUT1DS.
Glucose transporter type I deficiency syndrome (GLUT-1 DS) is a treatable epileptic encephalopathy due to a genetic disorder that impairs brain metabolism. The phenotype is variable ranging from severe impairment to children without seizure. Symptoms may include variable degrees of developmental delay, including speech and language disorders, epilepsy, spasticity, ataxia and a complex movement disorder usually with onset in childhood.

The Ketogenic diet (KD) should be the treatment of choice. There are several reports in literature about the effectiveness of KD in GLUT-1 DS in a short period but few reports assess the efficacy of a long-term treatment. A 2-5 years follow-up is reported by Klepper et al. in 2005 on 15 children, but is rarely offered to adults affected, because of the perceived inefficacy and restrictiveness.

We report our experience about a long-term KD follow-up of 6 years in three unrelated Italian GLUT-1 DS female patients, diagnosed in early adulthood, considering the effects of the ketogenic diet in term of clinical evolution, biochemical data, side effects and compliance.

Our illustrative cases were 3 females age ranged now 26–27 years old. We evaluated them from October 2006 to January 2013. Their clinical, biochemical, genetic features and the first year of follow-up have been described in details in our previous articles [Zorzi et al., 2008; Veggiotti et al., 2010].

Patients were evaluated at baseline, and every year during the follow up. At each time they underwent to a specific protocol including: neurological examination (NE), video-EEG monitoring and annually cognitive evaluation (Wechsler Adult Intelligence Scale). Metabolic screening including urine and blood analysis and ketone levels, abdominal ultrasounds were performed annually. Dietetic and nutritional evaluation [Veggiotti et al., 2011] were done every 3 months during the first year and once a year in the following. Dieticians worked out a normocaloric 3:1 (fat:non fat) long-chain triglyceride KD supplemented with sugarless multivitamins, calcium and potassium citrate, according to basal metabolic rate and daily physical activity level. Fluids were unrestricted and dietary plans were usually revised at each control. An accurate food diary was filled in by patients’ caregivers over the years.
- Clinical features

Clinical peculiarity, NE and EEG features at baseline are widely described in our previous chapter.

- Nutritional and laboratory features

At baseline two subjects were normal-weight and one was slightly under-weight (patient 3). The energy content of this subject’s diet was formulated in order to correct the energy balance. Routine laboratory investigations were unremarkable except for high total and LDL cholesterol level in patient 1, who was treated with policosanol supplement.

- Ketogenic meal plan

One of the major problematic issue concerned the appropriate fat/other nutrient ratio in order to achieve a good efficacy and to prolong the diet without significant adverse effects. The meal plan was a 3:1 KD ratio to obtain a right balance between energy and proteins requirements and the need to provide a satisfying taste to support patients’ compliance. A 4:1 ratio, described in literature for GLUT1-DS child with classic form, was too strict for our patients, considering the need to carry on the new diet as long as possible.
RESPONSE TO THE DIET IN LONG TERM FOLLOW-UP

Follow up data are summarized in table 6.

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
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<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T1</td>
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<td>Epileptic seizures</td>
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<td>-</td>
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<td>Paroxysmal dyskinesias</td>
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</tr>
<tr>
<td>AEDs</td>
<td>VPA</td>
<td>VPA</td>
</tr>
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</table>

Table 6

T0: baseline; T1: 1 year after the introduction of KD; T3: 3 years after the introduction of KD; T5: 5-6 years after the introduction of KD.

+: Present; - : absent; PNKD: Paroxysmal Non-Kinesigenic Dyskinesias; PED: Paroxysmal Exercise-induced Dyskinesias; Task 1*: maintaining a postural position of Mingazzini I and II; Task 2**: sitting and standing from a chair (number of times); Task 3***: climbing and descending a series of 10 steps (number of times); Task 4****: deambulation.

Ketogenic diet type: 3:1: classical 3 gr lipids:1gr charbohydrates- Betaidrossibutirrate: mean value of measuremets during the year (one survey per month)

This 6 years table in almost superimposable to our previous paper’s table. This support our assertion that efficacy of the diet persisted over several years.
- Clinical features

With the onset of ketosis all patients showed a rapid and complete disappearance of paroxysmal dyskinesias (PED). Then a progressive global resistance to physical effort was reported by patients and observed during following NE. Complex motor disorder consistent in spastic, ataxic and dystonic elements resulted moderately improved with variable degree in the 3 patients: Patient 1 showed only a mild reduction of dystonic movements; Patients 2 and 3 showed a clear improvement in dysarthric speech and some fine motor skills. There was no clear improvement in cognitive impairment in all cases, anyway parents reported an increase in alertness and activity.

- EEG

In childhood all our patients had presented seizures, treated with AEDs. In adolescence seizures disappeared, we do not know whether due to drugs or to the natural history of the disease. However, each one has continued with AEDs also in adulthood because of the persistence of important EEG epileptiform discharges. With the introduction of KD (we treated them from late adolescence), EEG showed a significant and rapid improvement in background activity and organization in all cases. Further, epileptic discharges disappeared completely allowing discontinuing antiepileptic medications in all patients after 2 year of treatment (see table 6).

- Nutritional and laboratory features

The dietary plan was modified during the years with the introduction of new food choices in order to adapt the changing tastes and energy needs but without changing the original nutrient composition and ketogenic ratio. After 5-6 years on diet, weight and BMI of all patients were in normal range for age: patients 1 and 2 had a little lost of weight and patient 3, underweight at baseline, maintained her weight. Computerized Bone Mineralometry (CBM) found a moderate decrease in bone mineralization and it is discussed in detail elsewhere; body composition changed according to body weight. (manuscript in preparation)

Routine laboratory investigation generally remained normal: the patient with baseline abnormal total and LDL colesterol treated with policosanol normalized her levels. Abdominal ultrasounds remained normal.
- Side effects
In all patients KD was well tolerated: no significant adverse events [Veggiotti et al., 2011] were reported. We report only a mild elevation of blood uric acid detected in patient 1 at the beginning of the diet (after 3 months) and corrected with a higher liquid intake. Beside we report a progressive hair loss in patient 3 starting from 6 months of the diet and now stabilized.

- Compliance
Generally patients' compliance was high. In one case (patient 3) there was a short term (about 3 months) voluntary interruption of KD after the first 2 years of KD. The discontinuation brought to an immediate recurrence of characteristic paroxysmal phenomena and worsening in EEG path. After recovery and resumption of KD, the paroxysmal events disappeared after few days (figure 16).
Figure 16. Change in patient 3 EEG pattern during the ketogenic diet interruption (above) and after the reintroduction of the diet (below).
DISCUSSION

In GLUT-1DS, the metabolic defect causes an impaired transport of glucose across the blood-brain barrier and leads to a loss in the brain basic energy supply that results in a heterogenic phenotype of epileptic encephalopathy. In these patients KD is an irreplaceable treatment that can provide an alternative fuel to the brain through the ketone bodies [Brockman, 2009]. To be effective the diet has to be followed for the rest of the affected lives.

In our experience of a long-term follow-up lasting 5-6 years of treatment with KD in 3 GLUT-1DS adult patients the most striking result was the disappearance of the movement disorders since the first days, and than never reappeared during the diet (specially paroxysmal dyskinesias and reduction in strength level). This result determined a radical improvement in the quality of life of subjects who became able to move and walk without restraint and not disturbed by the onset of paroxysmal sudden movements. It was also possible to record an important improving in EEG epileptic discharge and background activity that progressively became normal and allowed us to discontinue all the AEDs. [Von Moers et al., 2002; Tazadok et al., 2013] (table 1).

Other neurological features consisting in a combination of spastic, ataxic and dystonic elements resulted only moderately improved with variable degree in our 3 patients, and difficult to document. We currently have no evidence that an earlier start of the diet could improve these symptoms. As suggested in previous literature [Wang et al., 2005], there was no evidence of a significant cognitive improvement. Since mental retardation represents an element of the syndrome, we may suggest that a late therapeutic intervention might have no effect on this complex function. However what we could document was a subjective feeling of increase of alertness and activity.

In conclusion there are limited data about effectiveness of KD in adolescent and adult. In a recent review [Payne et al., 2011] it has been demonstrated that when users are compliant, the KD can be effective in treating adolescent and adults with drug resistant epilepsy. Alongside these assertions, from our experience we can confirm the effectiveness of KD in young adult GLUT1DS. Our experience in GLUT1DS patients underlines that it is important to continue KD also in adult age to improve the quality of life in term of disappearance of PED, epileptic discharges and AEDs discontinuation. To avoid a loss of compliance in adolescents and adults, the Modified Atkins diet or Medium Chain Triglicerid KD could
be more palatable formulations prescribed [Payne et al., 2011]. In our opinion, it is important to offer patients the tasty and pleasant food as possible. We believe that the maintenance of adequate ketosis around 2-3 mmol/l is sufficient to ensure the effectiveness of the diet. Our experience is about a 3:1 diet that we observed as sufficient to reach an adequate ketosis in GLUT1 symptoms control and that is well tolerated and accepted even after 6 years of treatment. These data seem to indicate a steady and positive response to KD in adults. What is important in the future is collect more experience of long term follow-up, also in adults, and increase studies addressing to pathogenic mechanisms and potential new treatment strategies.
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