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GLUCOSE TRANSPORTER TYPE 1 DEFICIENCY DUE TO *SLC2A1* GENE MUTATIONS – A RARE BUT TREATABLE CAUSE OF METABOLIC EPILEPSY AND EXTRAPYRAMIDAL MOVEMENT DISORDER; OWN EXPERIENCE AND LITERATURE REVIEW*

DEFICYT TRANSPORTERA GLUKOZY TYPU 1 ZALEŻNY OD MUTACJI W GENIE *SLC2A1* – RZADKA ALE MOŻLIWA DO LECZENIA PRZYCZYNA PADACZKI METABOLICZNEJ I ZABURZEŃ POZAPIRAMIDOWYCH; DOŚWIADCZENIA WŁASNE I PRZEGLĄD PIŚMIENNICTWA

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Abstract

The aim: *To present the molecular and clinical characteristics of three children with glucose deficiency syndrome, an inborn rare metabolic disease, caused by mutations in the SLC2A1 gene.*

Material and methods: *The investigation was carried out in three children: two girls and one boy showing symptoms of GLUT1 deficiency syndrome (GLUT1-DS). They were referred for SLC2A1 gene analysis.*

Results: *The presence of mutations in all of them was confirmed. Only point mutations were identified, two missenses p.Gly132Ser, p.Arg212Cys and amino acid insertion p.Ser_Val227insValProPro. In two cases the mutations arose de novo, one was heritable of paternal origin.*

Conclusions: *GLUT1–DS shows high clinical variability. It should be suspected in children of any age presenting with single features or a combination of any form of intractable epilepsy with seizures of various types, movement disorders and paroxysmal events, especially triggered by exercise, exertion, or fasting, and any unexplainable neurological deterioration. The basic diagnostic hallmarks of GLUT1-DS are CSF hypoglycorrhachia and lowered CSF/Blood serum glucose ratio. This is why lumbar punction should be considered more frequently than it is in practice being performed nowadays. Antiepileptic drug treatment may be ineffective or even potentially detrimental. Early identification and molecular confirmation of GLUT1-DS is important, because this is a metabolic disorder and patients should as soon as possible primarily be treated with a ketogenic diet.*

Key words: GLUT1 deficiency syndromes, GLUT1-DS, refractory epilepsy, ketogenic diet, *SLC2A1*, phenotypical heterogeneity

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Streszczenie

Cel pracy: *Prezentacja wyników badań klinicznych i genetycznych u trzech pacjentów z zespołem niedoboru glukozy, rzadkiego schorzenia metabolicznego, powodowanego mutacjami genu SLC2A1*

Materiał i metody: *Badania przeprowadzono u 3 dzieci, 2 dziewczynek i jednego chłopca, u których stwierdzono występowanie objawów klinicznych wskazujących na zespół niedoboru transportera glukozy (ang. GLUT1 deficiency syndrone; GLUT1-DS). Pacjenci zostali skierowani na badanie molekularne genu SLC2A1.*

Wyniki: *Potwierdzono u wszystkich, obecność patogennych mutacji. Zidentyfikowano tylko mutacje punktowe powodujące zmiany typu missens p.Gly132Ser, Arg212Cys i insercję aminokwasów p.Ser_Val227insValProPro. W dwóch przypadkach mutacje miały charakter de novo, jedna została odziedziczona od ojca*

Wnioski: *Zespół GLUT1-DS charakteryzuje się dużą zmiennością fenotypową. Chorobę tę należy podejrzewać u dzieci w każdym wieku, u których występuje lekooporna padaczka z polimorficznymi napadami i/lub różne typy zaburzeń ruchowych i napadowych, w szczególności gdy są one prowokowane przez ćwiczenia, wysiłek, lub występują na czczo, a także w przypadku niewytłumaczalnego pogorszenia stanu klinicznego. Podstawnym markerem diagnostycznym jest tutaj obniżone stężenie glukozy w płynie mózgowo-rdzeniowym i niski indeks glukozowy. Z tego powodu wykonanie punkcji lędźwiowej należy rozważać częściej niż ma to obecnie miejsce w codziennej praktyce lekarskiej. Leki przeciwpadaczkowe mogą być nieskuteczne, a nawet potencjalnie szkodliwe. Wczesne rozpoznanie GLUT-1 DS i potwierdzenie molekularne tej rzadkiej wrodzonej choroby metabolicznej jest niezwykle ważne, gdyż pacjenci powinni być jak najwcześniej leczeni dietą ketogenną.*

Słowa kluczowe: zespoły niedoboru transportera glukozy GLUT1, GLUT1-DS, padaczka lekooporna, dieta ketogenna, *SLC2A1*, heterogenność fenotypowa

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INTRODUCTION

Epilepsy is a disorder characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition [1]. More than 50 million people worldwide suffer from epilepsy, and in up to a third of them, seizures fail to respond to antiepileptic treatment. While such patients represent the minority of the population with epilepsy, they require an overwhelming amount of time, effort, and focus from the physicians who treat them. Refractory epilepsy may be progressive, carrying risks of structural damage to the brain and nervous system, comorbidities (osteoporosis, fractures), and increased mortality (accidents, sudden unexpected death in epilepsy), as well as psychological, educational and social consequences (stigma) [2]. It is, therefore, important to undertake any effort to diagnose potentially treatable disorders. GLUT1-DS together with *SCN1A*-related epilepsy disorders constitute rare genetic disorders in which molecular diagnosis may lead to therapy optimization [3].

Glucose is the primary energy source meeting the metabolic demand of the human brain. The main glucose transporters in the brain are GLUT1 and GLUT3. Transport across the blood-brain barrier (BBB) is performed exclusively by the transmembrane isoform of GLUT1 expressed in endothelial cells (the second isoform of transporter is expressed in astrocytes) [4]. The GLUT1 protein is encoded by the *SLC2A1* gene (MIM 138140) localised on chromosome 1p34 [5, 6]. Heterozygous mutations in this gene cause GLUT1 deficiency syndrome type 1 (GLUT1-DS1, MIM606777). The susceptibility to idiopathic generalized epilepsy-12 (EIG12, MIM 614847) is also conferred by mutations in the *SLC2A1* gene [5, 7]. GLUT1 deficiency syndrome (GLUT1-DS) was first described in 1991 by De Vivo et al [8]. They presented two infants with refractory epilepsy, psychomotor regression, acquired microcephaly and ataxia. As the patients showed low CSF glucose and lactate values in the absence of hypoglycemia, a pathomechanism based on a defect in glucose transport across the BBB was suggested [8]. Impaired glucose transport results in low cerebrospinal fluid (CSF) glucose levels called hypoglycorrhachia. However, this classical phenotype of early-onset epileptic encephalopathy now called GLUT1-DS1 rapidly expanded [10]. Now it is known that *SLC2A1*-related disorders show more complex clinical phenotypes, including a spectrum of different epilepsies and epileptic syndromes with ataxia, headaches, and cognitive impartment, but also complex movement disorders, like dystonia type 9 (DYT9, MIM 601042) and 18 (DYT18 known also as GLUT1-DS2, MIM, 612126), including choreoathetosis and paroxysmal exercise-induced dyskinesia, alternating hemiplegia, pyramidal paresis with or without epileptic seizures [11]. The clinical severity of *SLC2A1*-related

disorders varies from mild cognitive and motor dysfunction to severe neurological disability. Some patients never achieve language skills or unsupported walking. The diagnostic hallmark of the GLUT1-DS is impaired glucose transport into the brain – hypoglycorrhachia in a setting of normoglycaemia, so lumbar punction (LP) is the most important test for the diagnosis of those syndromes. Lumbar punction should be performed in a metabolic steady state, achieved after 4-6 hours of fasting and the blood glucose concentration should be checked before LP in order to avoid hyperglicaemia related to stress [12]. CSF lactate concentration should also be determined. The latter level is low – to normal, and this differentiates GLUT1-DS from other causes of hypoglycorrhachia. The diagnosis of GLUT1-DS is established in neurologically affected subjects with a disturbed ratio of glucose CSF/blood serum. Detection of the heterozygous mutations in the *SLC2A1* gene confirms this diagnosis. GLUT1-DSs are metabolic syndromes, so the proper diagnosis makes it possible to introduce effective treatment to patients. At present a ketogenic diet is regarded as the treatment of choice in most cases of this condition, as in this high –fat diet, ketone bodies bypass the GLUT-1 defect [13].

Here we present patients with GLUT1-DSs, for whom the diagnosis was confirmed by identification of mutations in the *SLC2A1* gene.. They presented a different clinical picture of the disease encompassing epilepsy, as well as the extrapyramidal movement disorder. The aim of this paper is to discuss the problems of such clinical heterogeneity, reduced penetrance of the mutations and therapeutic strategies also in the context of the introduction of the ketogenic diet.

MATERIAL AND METHODS

Our investigations were carried out in three children (2 girls and 1 boy) showing symptoms of GLUT1-DS, referred for the *SLC2A1* gene analysis, and included clinical and molecular characteristics of the patients presented. Clinical assessment included: the age of the first symptoms and their characteristics, the neuropsychological status, the type of seizures and epileptic syndrome, electroencephalographic, biochemical and neuroimaging findings, as well as movement disturbances. The molecular analysis involved the *SLC2A1* gene analysis.

1. Clinical assessment:

Patient 1 (*SLC2A1* genotype - c.[634T>C];[=], heterozygous mutation p.Arg212Cys)

This patient, currently aged 12, was hospitalized for the first time in our clinic department due to status epilepticus of generalized tonic-clonic seizures (GTCS) in his 3rd year of life. His father suffered from schizophrenia. He was born from an uneventful third pregnancy and had vaginal delivery at term with the weight of 3700g, length 57cm, head circumference 35 cm, and 9 points in the Apgar score. Early psychomotor development and neurological examinations were normal. At the age of 2 years he used to fall on the ground during games involving running. On admission, his physical growth was appropriate for the age, with head circumference 50 cm (75 percentile). Neuropsychological assessment showed incorrect eye-hand coordination and frontal lobe dysfunction (perseverations and inertia of reactions). Brain MRI indicated a slight delay of myelinization. Focal spikes in frontal areas and 4 Hz generalized spike-and-wave complexes were found in sleep EEG. Polymorphic seizures -GTCS, myoclonic-astatic, tonic – were observed, so we were able to diagnose Myoclonic-Astatic Epilepsy (MAE). Myoclonic seizures increased during Phenobarbital (PB) treatment. Valproate (VPA) administration was successful. He was re-admitted to our clinic after a few months, because of recurrent seizures. Furthermore, during fever or infections, ataxia and bradykinesia were observed. Metabolic tests for inborn errors of metabolism were negative. Topiramate (TPM) was added and seizure control improved. CSF glucose concentration was 34mg/dl with CSF-blood-glucose ratio: 0.57. The mutation in the *SLC2A1* gene was identified, and its paternal origin confirmed, when the patient was 10 years old. His mother refused the introduction of the ketogenic diet (KD). The patient remained seizure-free with normal EEG traces on VPA and TPM treatment until 11 years of age. Next time treatment was withdrawn and he experienced a few tonic seizures, all of them before breakfast after long sleep lasting over 12 hours. His cognitive development was delayed from his sixth year of life and he presented with mild intellectual disability. Head growth decelerated with a circumference of 53 cm (10 percentile), and he presented with mild ataxia (tab. I).

Patient 2. (*SLC2A1* genotype c.[388G>A];[=], heterozygous mutation p.Gly132Ser)

Currently a 5-year-old girl, has negative family history. She was born at term after an uneventful pregnancy and had vaginal delivery as the second child with the weight of 3850 g, head circumference 34 cm, and 10 points in the Apgar score. At the age of 4 years she experienced two episodes: at 5 a.m. she woke up crying, complaining of abdominal pain and displaying left-sided dystonic posture lasting half an hour, ending with vomiting. Her consciousness was preserved. At that time her EEG trace during sleep showed generalized spikes-and-polyspikes and wave complexes. Epilepsy was diagnosed but her parents refused to introduce antiepileptic treatment. Similar episodes repeated several times, always while fasting. Once before lunchtime, such an episode ended after the consumption of a sweet pear. During fasting she also experienced episodes of tiredness, slurred speech and loss of consciousness lasting a few seconds. She was admitted to our clinic at the age of 4.5 years. Her physical growth was appropriate for the age: height 110 cm (75th percentile), weight 18.5 kg (50th-75th percentile), and head circumference 50 cm (25th-50th percentile). Neurologic examination was unremarkable. Her cognitive level was within normal range (Leiter scale IQ =96). Attention deficits and eye-hand coordination deficits were observed. MRI of the brain was normal. Sleep and waking EEG examination revealed generalized spikes, polyspikes, sharp waves and slow wave complexes. Based on the history of the disease we suspected GLUT1

Table I. Molecular and clinical characterization of the presented patients with mutations in the *SLC2A1* gene.
Tabela I. Charakterystyka kliniczna i molekularna pacjentów, u których zidentyfikowano mutacje w genie SLC2A1.

Patient number ***Liczba pacjentów***	**1**	**2**	3
Sex ***Płeć***	M M	F K	F K
Age of first symptoms ***Wiek wystąpienia pierwszych objawów***	2 years *2 lata*	3 years *3 lata*	6 weeks *6 tygodni*
First symptoms ***Pierwsze objawy***	Astatic seizures *Napady astatyczne*	Dystonia and/or paresis left-sided with vomiting *Dystonia i/lub niedowład lewostronny z wymiotami*	Myoclonic and right side or generalized tonic *Napady mioklonicze oraz toniczne prawostronne lub uogólnione*
Neuropsychological assessment before DK (years) ***Badanie neuropsychologiczne przed włączeniem DK (wiek w latach)***	IQ 90 (3) IQ 62 (11)	Normal psychomotor development *Prawidłowy rozwój psychoruchowy*	Normal psychomotor development *Prawidłowy rozwój psychoruchowy*
Head circumference (at birth) ***Obwód głowy (przy urodzeniu)***	Normal *Prawidłowy*	Normal *Prawidłowy*	Normal *Prawidłowy*
Epilepsy ***Padaczka***			
Types of seizures ***Typy napadów***	Myoclonic-astatic, GTCS, Tonic with autonomic symptoms *Miokloniczno-astatyczne, uogólnione toniczno-kloniczne, toniczne z objawami wegetatywnymi*	Absences *Napady nieświadomości*	Myoclonic, tonic, atonic, clonic *Miokloniczne, toniczne, atoniczne, kloniczne*
EEG (age) ***EEG (wiek)***	Generalized spike-wave complexes and focal spikes (3 years) *Uogólnione wyładowania zespołów iglica-fala oraz zmiany zlokalizowane pod postacią iglic (3 lata)*	Generalized spikes and polyspikes-waves complexes (4 years) *Uogólnione wyładowania iglic i zespołów iglica –fala (4 lata)*	Normal (2 month); Generalized spike-waves 2-3Hz (2 years) *Prawidłowe (2 miesiące); Uogólnione wyładowania zespołów iglica – fala (2 lata)*
Epileptic syndrome ***Zespół padaczkowy***	Myoclonic-astatic epilepsy *Padaczka miokloniczno-astatyczna*	Epilepsy with absences of early onset *Padaczka z napadami nieświadomości o wczesnym początku*	Unclassified *Niesklasyfikowany*
Movement disorders ***Zaburzenia ruchu***			
Dystonia *Dystonia*	No *Nie*	Yes *Tak*	No *Nie*
Ataxia *Ataksja*	Yes *Tak*	No *Nie*	No *Nie*
Choreoathetosis *Choreoatetoza*	No *Nie*	No *Nie*	Yes *Tak*
Spastic paraplegia *Niedowład spastyczny kończyn dolnych*	No *Nie*	No *Nie*	Yes *Tak*

Table I. Cont.

Tabela I. Cd.

Glucose measurements ***Ocena stężenia glukozy***			
CSF glucose (mg/dl) *Glukoza w PMR (mg/l)*	34	36	39
Blood glucose (mg/dl) *Glukoza we krwi*	59	81	80
CSF/blood glucose ratio *Wskaźnik glukozowy PMR/krew*	0,57	0,44	0,49
Lactate measurements ***Ocena stężenia mleczanów***			
CSF lactate *Mleczany w PMR*	NP NB	1,04	NP NB
Molecular diagnosis ***Diagnostyka molekularna***			
Age at molecular diagnosis *Wiek rozpoznania*	10 years *10 lat*	4.5 years *4.5 lat*	5 years *5 lat*
SLC2A1 mutation *Mutacja SLC2A1* DNA *SLC2A1* gene localization *Lokalizacja genu SLC2A1* Protein *Białko* GLUT1 protein localization *Lokalizacja białka GLUT1***	c.[634T>C];[=], Exon 5 p.[Arg212Cys];[=] Intracellular loop *pętla wewnątrzkomórkowa*	c.[388G>A];[=], Exon 4 p.[Gly132Ser];[=] 4th transmembrane helix *4th transbłonowa helisa*	c.[680-11G>A];[=], Exon 5 p.[Ser226_Val227 insValProPro];[=] Intracellular loop *pętla wewnątrzkomórkowa*
Mutation inheritance *Dziedziczenie mutacji*	paternal *ojcowskie*	*de novo* nowa mutacja	*de novo* nowa mutacja
Family data *Wywiad rodzinny*	Father – schizophrenia *Ojciec – schizofrenia*	No *Nie*	No *Nie*
Treatment ***Leczenie***			
Ketogenic diet introduction – age *Wprowadzenie diety ketogennej – wiek*	No *Nie*	5 years *5 lat*	4 years *4 lata*
Ketogenic diet outcomes *Efekty leczenia dietą ketogenną*	Not introduced *Nie wprowadzono diety*	Improvement *Poprawa*	6 months treatment: seizures reduction, walk improvement, aggression and abnormal behaviour increased *leczenie przez 6 miesięcy: redukcja napadów, poprawa chodu, nasilenie agresji i zaburzeń zachowania*

Table I. Cont.

Tabela I. Cd.

Current status ***Obecny stan zdrowia***			
Patient's age *Wiek pacjentów*	12years *12 lat*	6years *6 lat*	7years *7 lat*
Epilepsy in remission *Remisja padaczki*	No *Nie*	Yes *Tak*	No *Nie*
Movement disturbances *Zaburzenia ruchu*	Yes *Tak*	No *Nie*	Yes *Tak*
Final diagnosis ***Ostateczne rozpoznanie***	GLUT1-DS1 Refractory Myoclonic-Astatic Epilepsy GLUT1-DS1 *Padaczka Mioklonicznо-Astatyczna lekooporna*	GLUT1-DS2/DYT18 Epilepsy with Early-Onset Absences GLUT1-DS2/DYT18 *Padaczka z napadami nieświadomości o wczesnym początku*	DYT9 Refractory Epilepsy with Polymorphic Seizures DYT9 *Padaczka lekooporna z napadami polimorficznymi*

Abbreviations: GLUT1-DS1 – Glucose transporter 1 deficiency syndrome type 1; DYT9 – Dystonia type 9; Glut1-DS2/DYT18 – Glucose transporter 1 deficiency syndrome type/Dystonia type 18 (Paroxysmal choreoathetosis/spasticity); GTCS – General Tonic-Clonic Seizures; CSF – Cerebrospinal Fluid; DK – Ketogenic diet ; NP – not performed

Skróty: GLUT1-DS1 – zespół niedoboru transportera glukozy GLUT1 typ 1; DYT9 – dystonia typu 9; GLUT1-DS2/DYT18 – zespół niedoboru transportera glukozy GLUT1 typ2/dystonia typu 18 (napadowa choreoatetoza/spastyczność); PMR – płyn mózgowo-rdzeniowy; NB – nie badano

deficiency syndrome with movement disorders (dystonia) and Early-Onset Absence Epilepsy. In fact we found CSF hypoglycorrhachia (tab. I). Molecular findings confirmed our diagnosis. We advised putting the child on KD and introducing VPA. Due to the parents' disagreement only the ketogenic diet was conducted, with cessation of paroxysmal episodes.

Patient 3. (*SLC2A1* genotype c.[680-11G>A];[=], heterozygous mutation p.Ser226_Val227insValProPro)

Currently a 7-year-old girl, born from a second, normal pregnancy, on time, weighing 3260 grams, with head circumference 37 cm and the Apgar score of 10. There was a negative genetic family history. She experienced myoclonic and right side or generalized tonic seizures 2 days after vaccination, at 6 weeks of life. EEG and head USG was normal. Phenobarbital was first introduced. Due to persistent myoclonic seizures, valproic acid was added at 3 months of age. Seizures remitted at 5 months of age, but development regression began (crawling at 18 months of age, trying to walk with help at 2 years of age, she did not speak). Myoclonic and atonic seizure recurrence was noted at 2 years of age and at 4 years of age nocturnal generalized clonic seizures were observed too. She was treated with lamotrigine and clobazam at that time. Levetiracetam and topiramate did not improve the course of the disease, short-lasting remission was achieved during synacthen. Abnormal gait, choreoatetotic movements in hands and feet, which exaggerated after exercises and between meals, were observed. Generalized spike-and-wave complexes of 2-3 Hz were found. Psychological examination showed global, significant developmental delay. CSF glucose level was 39 mg% (the next test showed: 42 mg%). The CSF-blood-glucose ratio was 0.49. The ketogenic diet was introduced for the next 6 months. Significant seizure reduction and gait improvement was observed, but there were behavioral problems and aggression – her mother decided to stop the ketogenic diet. At 5 years of age there are still numerous seizures: myoclonic, atonic, and nocturnal clonic. Gait with help, spastic paraplegia, dyskinesia within upper and lower extremities, acquired microcephaly (49 cm, increase only 12 cm over 7 years), moderate mental retardation, and lack of active speech.

2. Molecular analysis

Mutations causative for GLUT1-DSs include point mutations in the *SLCA2A1* gene (81-89%) and gene rearrangements (11-14%). This is why the first step of the analysis is the gene sequencing, if no point mutations are identified, further quantitative analysis is performed. The *SLC2A1* gene was sequenced using the Sanger method. All coding exons and exon/intron boundaries of the gene were analysed (primers, PCR conditions on request). The variants were labelled according to the *SLC2A1* reference sequence NM_006516.2 (NCBI RefSeq;http://www.ncbi.nlm.nih.gov). Involvement of identified mutations in

clinical phenotypes was checked in the Human Gene Mutation Database Professional (HGMD Professional v.2015.2; http://www.biobase-international.com/product/hgmd). For the probands' relatives, only appropriate exons of the gene were sequenced. The rearrangements analysis of the *SLC2A1* gene was performed using the multiplex ligation-dependent probe amplification (MLPA) method with commercially available MLPA probemixes: P-138A *SLC2A1* (MRC-Holland).

Written informed consent was obtained from all participants (in the case of minors – from the patient's legally authorized representative).

RESULTS

The patients identified as *SLC2A1* mutation carriers were all diagnosed as GLUT1-DS. They displayed different types of epilepsy accompanied with movement disorders, as well as hypoglycorrhachia in CFS and a reduced CSF/blood glucose ratio (ranging from 0.44 to 0.57).

In all the cases the mutations were identified as heterozygous, in two patients they arose *de novo*, in one case the mutation was of paternal inheritance. Mutations were localised in exon 5 (substitution c.388 G>A), exon 4 (substitution c.634 T>C) and inton 5 (substitution IVS5 c.680-11G>A). The exonic substitutions caused missense mutations in the 4th transmembrane domain (p.Gly132Ser) and an intracellular loop (p.Arg212Cys) of the GLUT1 protein. Intronic mutation, which is localized -11 nucleotides upstream of the exon 6 splicing site, generates a new acceptor site and finally causes the insertion of additional amino acids (p.Ser_Val227insValProPro) in the intracellular loop connecting transmembrane domains 6 and 7 (fig.1).

All of those mutations were previously identified in GLUT1-DSs and classified as pathogenic (HGMD Professional 2015.2, https://portal.biobase-international.com/hgmd/pro/gene.php?gene=SLC2A1). All of them were described as causative for GLUT1-DS1, but insertion p.Ser_Val227insValProPro additionally for DYT9 (9,14-17).

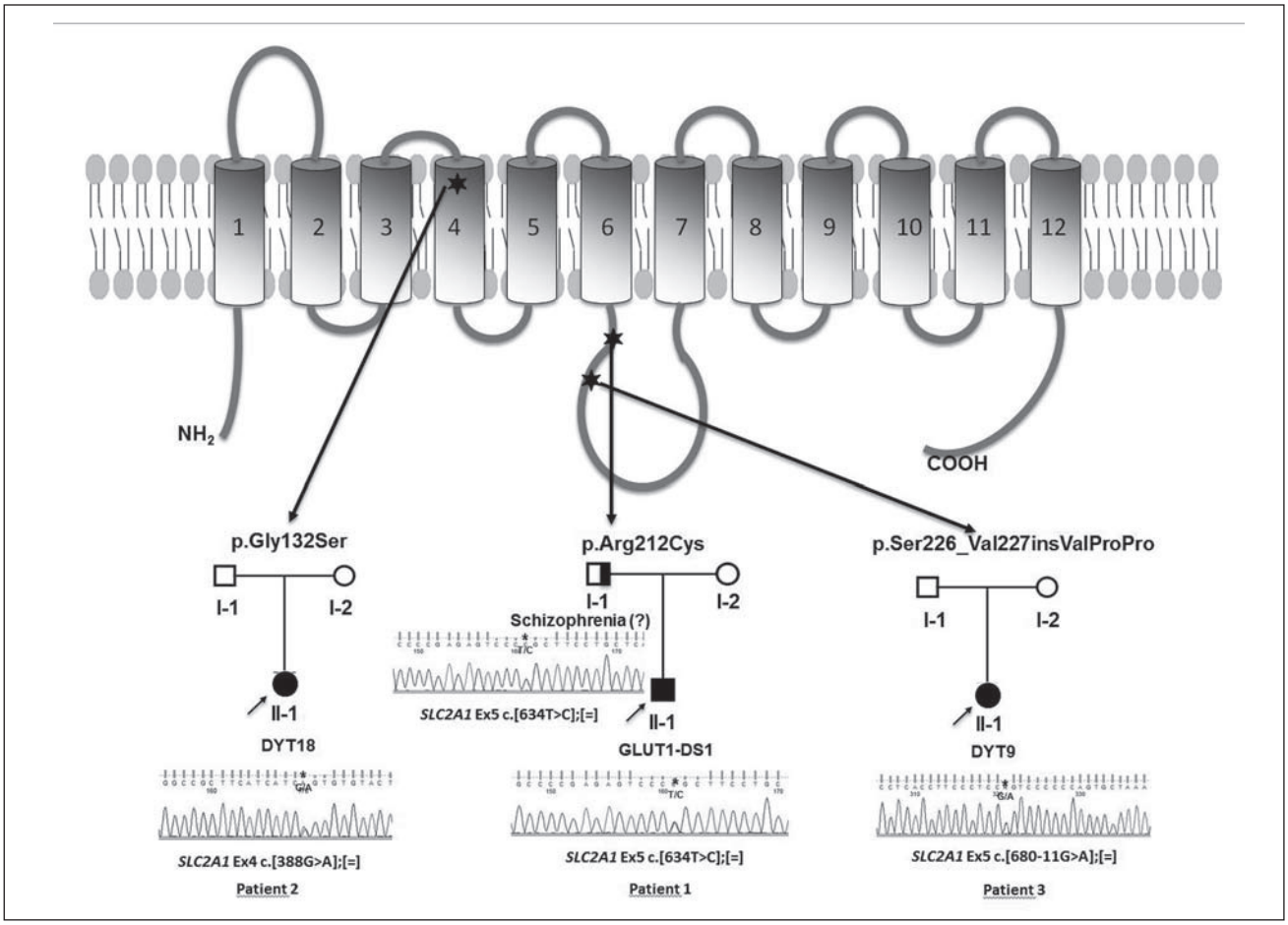

Fig. 1. Characterization of the identified SLC2A1 mutations from GLUT1-DS patients – all mutations resulted in the development of the GLUT1-DS1 phenotype. The locations of the mutations in the GLUT1 protein are marked. Pedigrees show the mode of inheritance. Only in one case the mutation was heritable, substitution p.Arg212Cys was passed from the proband's father, who suffers from schizophrenia.

Ryc. 1. Charakterystyka mutacji w genie SLC2A1 zidentyfikowanych u pacjentów z zespołem niedoboru transportera glukozy GLUT1 (GLUT1-DS). Zaznaczono lokalizacje poszczególnych mutacji na schemacie białka GLUT1. Rodowody wskazują na sposób dziedziczenia zidentyfikowanych mutacji. Tylko w przypadku substytucji p.Arg212Cys mutacja została odziedziczona od ojca ze zdiagnozowaną schizofrenią.

A summary of the clinical and molecular characterizations of the cases is presented in table I, localization of the mutations in the GLUT1 protein is presented in figure 1.

DISCUSSION

We have presented three patients from our epilepsy centre, with epilepsy due to *SCL2A1* mutations and would like to discuss some notable points:

Epilepsy phenotype. Epilepsy in GLUT 1 deficiency can start at any age, but seizures usually occur in infancy and early childhood, with the mean age of about 8 months [6, 11]. The onset of seizures in our patients was between the age of 6 weeks and 3 years. In our patients we observed seizures of different morphology. In fact, so far it seems that no specific seizure phenotypes are connected with this entity [18]. Seizures in infants are usually described as head bobbing, myoclonic limb jerks, cyanotic spells, eye-rolling or horizontal roving eye movements with diminishing alertness. Two of our patients had more than one seizure type. This is consistent with the study by Pong et al., who in the group of 87 children with GLUT1-DS observed that most of them had polymorphic seizures: GTCS, absences, complex partial seizures, myoclonic-astatic, drop, tonic seizures, and spasms [6]. These authors were the first ones to report infantile spasms in children with impaired function of GLUT1. Additionally, no characteristic pattern of EEG traces in GLUT1-DS has been described so far [6, 11, 18]. The EEG traces of infant patients usually show multifocal spike discharge. Subsequently, with brain maturation, seizures become synchronized and generalized with discharges of 3-4 Hz spike-wave complexes. Similarly to the types of seizures, it seems that no specific epilepsy phenotypes are connected with GLUT 1 deficiency. It has, however, been stated that some epilepsy phenotypes of GLUT1 deficiency syndrome may be masquerading as idiopathic generalized epilepsy syndromes (IGE) [19]. Lebon et al found 2.1% patients with GLUT1 in a group of 93 unrelated children with generalized epilepsy [20]. Gaspard et al reported one case of Begin Myoclonic Epilepsy of Infancy (BMEI) as the initial presentation of that entity [21]. However, according to Lebon et al. GLUT1-DS is most likely to be found in MAE and in Early-Onset-Absence Epilepsy (AOAE), but apart from seizures, these patients are likely to have additional neurologic symptoms, such as the slowing of head growth, cognitive impairment and movement disorders [20]. Both our patients (1. and 2.) with such epilepsy syndromes fit the above description. Those authors concluded that the probability of finding GLUT1 deficiency in the well-known phenotypes of idiopathic generalized epilepsy (only with seizures) is low.

In familial cases of GLUT1-DS (autosomal dominant of inheritance) phenotypic heterogeneity is observed. Suls et al. evaluated a five-generation family with 25 individuals being *SLC2A1* mutation carriers. They presented epilepsy, PED and both phenotype; 14 (56%), 19 (26%) and 11 (44%) respectively [22]. The co-occurence of PED and epilepsy suggested common clinical entity. Functional imaging studies showed alterations in glucose metabolism in the frontal lobe cortex in the pathophysiology of epileptic seizures, while in the corticostriate pathways in the pathophysiology of PED. In this family predominant epileptic seizure types were primary generalized: absences, GTCS, and complex and simple partial seizures, myoclonic (with myoclonic status epilepticus), and febrile seizures. One patient with partial seizure reported unformed visual hallucinations and a rising epigastric sensation and nausea followed by loss of consciousness with the dystonic posturing of one arm. In the other familial cases of IGE, Mullen et al. found epilepsy in 12 family members out of 15 *SLC1A2* mutation carriers [23]. The spectrum of epilepsy syndrome included Juvenile Myoclonic Epilepsy (JME), Childhood Absence Epilepsy (CAE), Juvenile Absence Epilepsy, Early-Onset Absence Epilepsy (EOAE), and also focal epilepsy. Some epileptic patients presented with PED. This study confirmed that (EOAE) seems to be quite typical of GLUT1-DS. Its phenotype is similar to CAE, except for the earlier age of onset, as absences starting before 4 years of age [12]. Probably the youngest onset of EOAE was described in a child with short-lasting (less than 3sec) absences and rare myoclonias starting at the age of 3 months of life [12, 19]. In another study performed by Giordano et al. carried out on a cohort of 33 children with absence epilepsy, with episodes starting before 3 years of life (age range 8-36 months), no *SLC2A1* mutation was identified in the 20 patients tested. In the authors' opinion they failed to identify the *SLC2A1* mutation, probably due to the fact that they did not study children with additional neurological findings other than epilepsy [24]. For patient 2 the absences, which started before the age of 4 years, did not present dominant symptoms, but the girl showed prominent movement disorders with autonomic signs. Taking into account long-lasting (about half an hour) later episodes involving one side of the body with prominent autonomic symptoms, the age of onset, and normal cognitive development with normal MRI of the brain, in differential diagnosis we had also considered Panayiotopoulos syndrome [25]. However, since the fits always took place before meals and during prolonged fasting, and symptoms were relieved after carbohydrate food intake, we were prompted to consider GLUT1 deficiency. Characteristic signs of GLUT1-DS is the possibility to provoke and/or increase symptoms when fasting and improvement after carbohydrate intake, thus reflecting the cerebral energy deficit [10]. Brockmann et al reported familial mutation *SLC2A1*carriers with GLUT1-DS in two boys with refractory epilepsy and significant cognitive impairment and their mother who intuitionally ate every 3 hours round the clock and their grandfather who worked as a manager and was served honey at bedside in the morning by his wife to improve his functioning [26]. Avoiding prolonged fasting is recommended in cases of not being put on DK [13].

Glucose measurements: the first seizures in patient 1 occurred at the age of 2.8 years of life – he was a boy with normal psychomotor development. GLUT-1 deficiency due to *SLC2A1* mutation was diagnosed when he was 10 years old. One may ask why in the light of low-glucose

concentrations in his CSF (34m/dl), the diagnosis of GLUT1 deficiency was reached so late. His CSF-to-blood glucose ratio was 0.57. This value at the time of analysis was classified as within normal range. Initially, the glucose ratio of 0.33-0.37 was set as the cut-off value for a diagnosis of GLUT1 deficiency in suspected patients. The patients' average glucose ratio is 0.33, however it ranges from 0.19- 0.46 [10, 12]. In time, the range of GLUT1-DS phenotypes increased and milder cases have been diagnosed, higher values of this ratio up to 0.59 (CSF glucose 60 mg/dl) are now being applied . Nowadays it has been established that normal CSF-to-blood glucose ratio is above 0.6 [6]. There is also agreement that the CSF-to blood glucose ratio is superior to the absolute CSF glucose level.

Inheritance. Among our patients, only in one case was the mutation inherited from the father who was seizure-free but suffered from schizophrenia (fig. 1). Of course we are not able to solve the problem to which extent the *SLC2A1* mutation is responsible for his phenotype (especially without glucose measures, which we do not have). Such an influence could not be excluded. We can speculate that the mutation is not fully penetrant or that he is a mosaic carrier, and mutation is not present in the tissues responsible for the phenotype.

Treatment/Ketogenic diet. Epilepsy in GLUT1 deficiency syndrome patients can be treated effectively with the ketogenic diet (10), which mimics the metabolic state of fasting but maintains ketosis by the utilization of nutritional fat rather than body fat. Ketone bodies readily penetrate the blood–brain barrier and in setting low CSF glucose concentration, ketones provide alternative fuel to the brain in the energy deficit syndrome. Treatment with a ketogenic diet may lead to progress in myelination reflecting the effectiveness of management [10, 27]. The delay in making the diagnosis may result in irreversible brain damage due to long-lasting hypoglycorrhachia [12]. Early identification of the background of the disease is very important in order to provide a child with an alternative energy source during the time of increased cerebral metabolism in the developmental period. Antiepileptic drug treatment may be ineffective or even potentially detrimental, as valproate, diazepam and phenobarbital impair the function of the GLUT1 transporter. Even when seizures ceased on anticonvulsant treatment, further cognitive and motor development may be abnormal, as we observed in patient 3. Introducing KD resulted in the cessation of both seizures and dystonic fits in patient 2.with AOAE, although follow-up comprises only a few months.

The mother of patient 1 refused to introduce KD till the age of 11 years, so in this case we were able to analyze the natural course of this energy depletion disorder of the brain. We want to stress that in spite of not being on KD, since the age of 3.5 years till the age of 11 years, he was seizure free, with normal EEG. It is worth underlining that even in patients who were not on the ketogenic diet the course of epilepsy can be quite benign and even self-limiting, which may lead to the delay of the proper diagnosis. Bovi et al reported a case of a 26-year-old woman who had suffered from partial epilepsy from the age of 22 months to 4 years and 10 months [13]. She was treated with Carbamazepine and Phenobarbital. The latter was stopped at the age of 6 years. At the age of 13 years the patient began to experience lower limb fatigue after prolonged walking, which in her adulthood was recognized as PED. Molecular diagnosis confirmed SLC1A2 mutation at the age of 22 years. Nevertheless, she was not put on KD due to the benign nature of this phenotype. The authors did not mention her intellectual state. Our patient 1 was exposed to a long-lasting hypoglycorrhachia, so in spite of a long period of seizure remission, his cognitive ability deteriorated from normal IQ at the age of 3 years to intellectual disability at the age of 11, and his head growth deteriorated.

CONCLUSIONS

GLUT1-DS is a treatable metabolic syndrome, rare among epilepsies, and because of that it is important not to overlook patients with this syndrome, so they can be treated properly with a ketogenic diet. Our data confirm that the clinical phenotype of GLUT1-DSs is mostly complex and should be suspected in children of any age presenting with single features or especially in the combination of any form of intractable epilepsy with seizures of various types, especially with early-onset absence epilepsy, global developmental delay, particularly in speech, complex movement disorders, paroxysmal events (seizures, movement disorders) triggered by exercise, exertion, or fasting, and any unexplainable neurological deterioration. The basic diagnostic hallmark of this syndrome is CSF hypoglycorrhachia and glucose ratio below 0.60. This is why lumbar punction should be considered more frequently than it is performed in practice nowadays. EEG findings may not add much to diagnosis. Antiepileptic drug treatment may be ineffective or even potentially detrimental, because it can impair the function GLUT1 transporter. Even when seizures ceased on anticonvulsant treatment further cognitive and motor development can be abnormal. That is why the earliest possible GLUT1 deficiency recognition is of paramount significance.

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REFERENCES

1. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia. 2005;46:470-472.
2. Laxer KD, Trinka E, Hirsch LJ, Cendes F, Langfitt J, Delanty N, Resnick T, Benbadis SR. The consequences of refractory epilepsy and its treatment. Epilepsy & Behavior. 2014;37:59-70.
3. Terczyńska I, Szczepanik E, Duszyc K, Górka P, Tataj R, Hoffman-Zacharska D. Genetic epilepsies with febrile seizures plus: clinical spectrum of Polish patients with SCN1A mutation – preliminary report. Dev Period Med. 2014;18(4):426-431.

4. Duelli R, Kuschinsky W. Brain glucose transporters: relationship to local energy demand. News Physiol Sci. 2001;16:71-76.
5. Wang D, Kranz-Eble P, De Vivo DC. Mutational analysis of GLUT1 (SLC2A1) in Glut-1 deficiency syndrome. Hum Mutat. 2000;16:224-231.
6. Pong AW, Geary BR, Engelstad KM, Natarajan A, Yang H, De Vivo DC. Glucose transporter type I deficiency syndrome: epilepsy phenotypes and outcomes. Epilepsia. 2012;53:1503-1510.
7. Striano P, Weber YG, Toliat MR, Schubert J, Leu C, Chaimana R, Baulac S, Guerrero R, LeGuern E, Lehesjoki AE, Polvi A, Robbiano A, Serratosa JM, Guerrini R, Nürnberg P, Sander T, Zara F, Lerche H, Marini C; EPICURE Consortium. GLUT1 mutations are a rare cause of familial idiopathic generalized epilepsy. Neurology. 2012;78:557-562.
8. 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. New Eng J Med. 1991;325:703-709.
9. Wang D, Pascual JM, Yang H, Engelstad K, Jhung S, Sun RP, De Vivo DC. Glut-1 deficiency syndrome: clinical, genetic, and therapeutic aspects. Ann Neurol. 2005;57:111-118.
10. Klepper J. GLUT1 deficiency syndrome in clinical practice. Epilepsy Res. 2012;100:272-277.
11. De Giorgis V., Veggiotti P.: GLUT1 deficiency syndrome 2013: current state of the art. Seizure. 2013;22:803-811.
12. Verrotti A, D'Egidio C, Agostinelli S, Gobbi G. Glut1 deficiency: When to suspect and how to diagnose? European. J Paed Neurol. 2012;16:3-9.
13. Bovi T, Fasano A, Juergenson I, Gellera C, Castellotti B, Fontana E, Tinazzi M. Paroxymal exercise-indused dyskinesia with self-limiting partial epilepsy: A novel GLUT-1 mutation with benign phenotype. Parkinson and Relat Dis. 2011;17:479-481.
14. Deng D, Xu C, Sun P, Wu J, Yan C, Hu M, Yan N. Crystal structure of the human glucose transporter GLUT1. Nature. 2014;150:121-125.
15. Leen WG, Klepper J, Verbeek MM et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. Brain. 2010;133:655-670.
16. Suls A, Dedeken P, Goffin K, Van Esch H, Dupont P, Cassiman D, Kempfle J, Wuttke TV, Weber Y, Lerche H, Afawi Z, Vandenberghe W, Korczyn A.D, Berkovic SF, Ekstein D, Kivity S, Ryvlin P, Claes LRF, Deprez L, Maljevic S, Vargas A, Van Dyck T, Goossens D, Del-Favero J, Van Laere K, De Jonghe P, Van Paesschen W. Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in SLC2A1, encoding the glucose transporter GLUT1. Brain. 2008;131:1831-1844.
17. Weber YG, Kamm C, Suls A, Kempfle J, Kotschet K, Schüle R, Wuttke TV, Maljevic S, Liebrich J, Gasser T, Ludolph AC, Van Paesschen W, Schöls L, De Jonghe P, Auburger G, Lerche H. Paroxysmal choreoathetosis/spasticity (DYT9) is caused by a GLUT1 defect. Neurology. 2011;77:959-964.
18. Leary LD, Wang D, Nordli DR Jr, Engelstad K, De Vivo DC. Seizure Characterization and Electroencephalographic Feature in Glut-1 Deficiency Syndrome. Epilepsia 2003;44 (5):701-707.
19. Roulet-Perez E, Ballhausen D, Bonafé L, Cronel-Ohayon S, Maeder-Ingvar M. Glut-1 deficiency syndrome masquerading as idiopathic generalized epilepsy. Epilepsia. 2008;49:1955-1958.
20. Lebon S, Suarez P, Alija S, Korff CM, Fluss J, Mercati D, Datta AN, Poloni C, Marcoz J-P, Campos-Xavier AB, Bonaf_L, Roulet-Perez E. When should clinicians search for GLUT1 deficiencysyndrome in childhood generalized epilepsies? Eur J Paed Neurol. 2015;19:170-175.
21. Gaspard N, Suls A, Vilain C, De Jonghe P, Van Bogaert P. "Benign" myoclonic epilepsy of infancy as the initial presentation of glucose transporter-1 deficiency. Epileptic Disord. 2011;13 (3):300-303.
22. Suls A, Mullen SA, Weber YG, Verhaert K, Ceulemans B, Guerrini R, Wuttke TV, Salvo-Vargas A, Deprez L, Claes LR, Jordanova A, Berkovic SF, Lerche H, De Jonghe P, Scheffer IE. Early-onset absence epilepsy caused by mutations in the glucose transporter GLUT1. Ann Neurol. 2008;66:415-419.
23. Mullen SA, Suls A, De Jonghe P, Berkovic SF, Scheffer IE. Absence epilepsies with widely variable onset are a key feature of familial GLUT1 deficiency. Neurology. 2010;75:432-440.
24. Giordano L, Vignoli A, Accorsi P et al. A clinical and genetic of 33 new cases with early-onset absence epilepsy. Epi Res 2011; doi.1016/j.eplepsyres.2011.03.017
25. Michael M, Tsatsou K, Ferrie CD. Panayiotopoulos syndrome: An important childhood autonomic epilepsy to be differentiated from occipital epilepsy and acute non-epileptic disorders. Brain & Dev. 2010;32:4-9.
26. Brockmann K, Wang D, Korenke CG, von Moers A, Ho YY, Pascual JM, Kuang K, Yang H, Ma L, Kranz-Eble P, Fischbarg J, Hanefeld F, De Vivo DC. Autosomal dominant glut-1 deficiency syndrome and familial epilepsy. Ann Neurol. 2001;50 (4):476-485.
27. Klepper J, Engelbrecht V, Scheffer H. GLUT1 deficiency with delayed myelination responding to ketogenic diet. Pediatr Neurol. 2007;37:130-133.

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