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Short communication

Three novel *SLC2A1* mutations in Bulgarian patients with different forms of genetic generalized epilepsy reflecting the clinical and genetic diversity of GLUT1-deficiency syndrome

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ABSTRACT

Purpose: GLUT1-deficiency syndrome (GLUT1-DS) is a metabolic brain disorder with a great clinical heterogeneity underlined by various mutations in the *SLC2A1* gene which make the clinical and genetic diagnosis complicated. The purpose of our study is to investigate the genetic defects affecting the *SLC2A1* gene in a group of Bulgarian patients with genetic generalized epilepsy (GGE), and to bring new insights into the molecular pathology of GLUT1-DS that would strengthen the genotype-phenotype correlations and improve the diagnostic procedure.
Methods: We have performed sequencing analysis of the *SLC2A1* gene in thirty-eight Bulgarian patients with different forms of GGE having emerged in childhood followed by array comparative genome (aCGH) hybridization in patients with severe forms of GLUT1-DS who display extraneurological features.
Results: We have detected three novel *SLC2A1* gene mutations that are predicted to have different impacts on the GLUT1 protein structure and function – one being to cause the amino acid substitution p.H160Q, another leading to the truncation p.Q360*, and also a 1p34.2 microdeletion. The overall frequency of the *SLC2A1* mutations in the studied group is 8.1%. They have been found in clinical cases that differ notably by their severity.
Conclusion: Our study enriches the mutation spectrum of the *SLC2A1* gene by 3 novel cases that reflect the genetic and phenotypic diversity of GLUT1-DS and brings new insights into the molecular pathology of that disorder. The clinical data showed that the *SLC2A1* genetic defects should be considered equally in the entire range of the clinical manifestations of GGE paying attention to the extraneurological features. The aCGH analysis should be considered as an ultimate step during the diagnostic procedure of GLUT1-DS in patients with a complex clinical picture of intractable epilepsy involving neuropsychological impairments and accompanied by extraneurological features.

1. Introduction

GLUT1-deficiency syndrome (GLUT1-DS) is a metabolic brain disorder with a major biochemical characteristic of hypoglycorachia caused by various mutations in the gene *SLC2A1* which encodes the primary glucose transporter protein in the brain,

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GLUT1 (glucose transporter 1) [1–3]. GLUT1 is a transmembrane protein creating a water-filled pore that transfers the glucose molecule across the blood-brain barrier [3].

SLC2A1 mutations have been found in a variety of epilepsy phenotypes ranging from the complex syndrome of 'classic' GLUT1-DS comprising infantile seizures, developmental delay, acquired microcephaly, hypotonia, spasticity and a complex movement disorder – to much milder forms of genetic generalized epilepsy (GGE) presented with absence only [3–5]. The great clinical heterogeneity emphasized by various genetic defects in the *SLC2A1* gene has made the clinical and genetic diagnosis of GLUT1-DS complicated.

Herein, we present the results from the molecular genetics study of the *SLC2A1* gene in Bulgarian patients with different forms of GGE having emerged in childhood. We discuss their molecular, clinical and diagnostic implications, and interpret them in terms of up to date data.

2. Materials and methods

Thirty-eight unrelated Bulgarian patients have been included in the study. Patients have been selected for the study based on the negative *SCN1A* mutation screening and the epilepsy phenotypes related to *SLC2A1* gene mutations that have already been described in the literature. Clinical phenotypes have been assessed following the International League Against Epilepsy (ILAE) Guidelines [6]. Amongst them, 8 patients displaying early-onset absence epilepsy (EOAE), 8 – childhood absence epilepsy (CAE), 8 with epilepsy of myoclonic and atonic seizures (EMAS), and 7 with epilepsy with myoclonic absences (EMA). Six patients have had unspecified GGE presented with generalized tonic-clonic seizures (GTCS) and absences, and one patient has displayed polymorphic seizures. Severe forms with mental retardation and accompanied by extraneurological features have been observed in 9 patients. A panel of 100 ethnically matched control DNA samples from healthy individuals has been tested for each novel sequence variant observed.

All procedures in this study involving human participants are in accordance with the ethical standards of the Medical University – Sofia and/or the National Research Committee. Informed consent has been obtained from all individual participants and/or their legal guardians.

DNA has been extracted from peripheral blood. Sequence analysis of all 10 exons and exon–intron borders of the *SLC2A1* gene has been performed using polymerase chain reaction (PCR) followed by Sanger sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®, LifeTechnologies). Sequences have been analyzed by ABI Sequencing Analysis v5.3

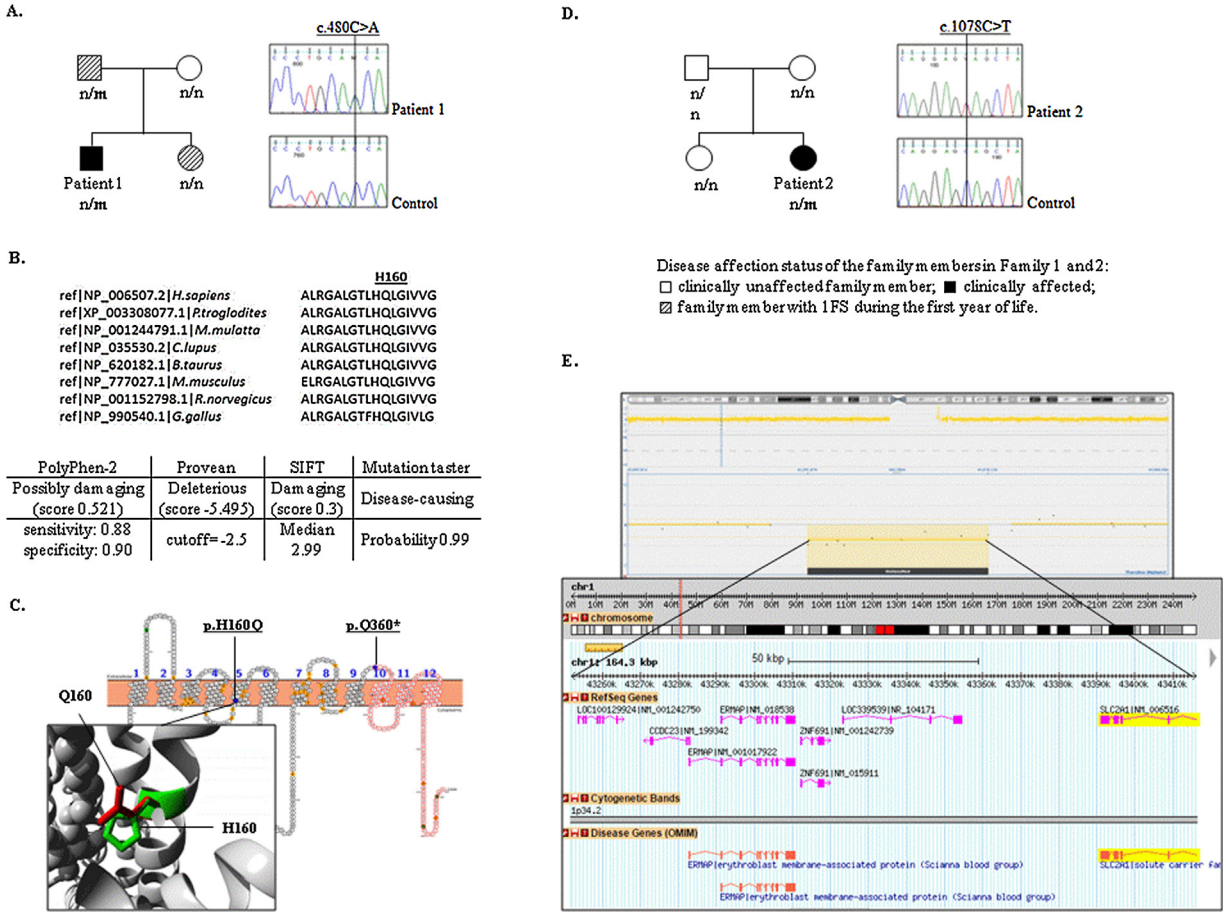

Fig. 1. *SLC2A1* genetic defects found in Bulgarian patients with suspected GLUT1-DS. A. Pedigree of Family 1 showing the carrier status of the family members and the electropheregram of the sequences comprising the mutation c.480C ≫ A in Patient 1 and a healthy control. B. Comparative analysis of GLUT1 orthologous proteins in ClustalW2 (http://www.ebi.ac.uk/) in the region of p.H160Q and *in silico* prediction of the effect of this amino acid substitution. C. GLUT1 membrane topology with mutations p.H160Q and p.Q360*, annotated by Protter (http://wlab.ethz.ch/protter/), and the 3D-structure of the GLUT1 protein generated in HOPE Project (http://www.cmbi.ru.nl/) using PDB-file 4PYP as a modeling template – close-up at the site of the mutation with both the referent and the mutated amino acid residue. D. Pedigrees of Family 2 showing the mutation carrier status of the family members and the electropheregram of the sequences comprising the mutation c.1078C ≫ T in Patient 2 and a healthy control. E. Microdeletion 1p34.2 chr1: g.(43251879-43416139)del (NCBI Build hg19:GRCh37:Feb 2009) with the gene content of the deleted region visualized on Database of Genomic Variants (http://dgv.tcag.ca/dgv/app).

software and compared with the reference NM_006516 (http://www.ncbi.nlm.nih.gov). All sequence variants have been checked on the Exome Variant Server and in the ExAc database (exac.broadinstitute.org/). Their effect on the GLUT1 structure and function has been predicted *in silico* by PolyPhen-2 (http://genetics.bwh.harvard.edu/), Provean (provean.jcvi.org/) and SIFT (sift.jcvi.org/). The pathogenicity has been evaluated by Mutation taster (www.mutationtaster.org/).

Comparative genomic hybridization assay (aCGH) using SurePrint G3 Human CGH Microarray Kit, 4 × 180 K (Agilent Technologies, Santa Clara, CA, USA) has been performed in patients with severe forms of GLUT1-DS who displayed neuropsychological impairments and extraneurological features. Copy number variations (CNV) have been confirmed and the inheritance has been tracked by Real-Time PCR analysis on ABI7300 workstation using following primers: Forward: 5′-CAGGCTTCGTGCCCATGTAT-3′ and Reverse: 5′-ACAGATCCGAGAGCCACTGA-3′.

3. Results

Sequencing analysis has revealed two novel single nucleotide substitutions. The first one, c.480C ⋙ A leads to the amino acid substitution H160Q in the cytoplasmic part of the TM5 segment in the GLUT1 protein (Fig. 1A, C). It affects evolutionary conserved amino acid and has been predicted *in silico* to be damaging and disease-causing (Fig. 1B). The 3D structure of the mutant and native GLUT1 has shown that the affected amino acid p.H160 has been exposed on the polar water-accessible face of the channel and the side chain of the mutant amino acid p.Q160 has been shifted compared to that of the native residue (Fig. 1C). The c.480C ⋙ A sequence variation has been found in Patient 1 (Fig. 1A), a 10-year old boy with electro-clinical data for CAE/JAE who responded well to the conservative treatment (Table 1). He was born after normal pregnancy and has had a normal neuro-psychological development. Segregation analysis of c.480C ⋙ A in the family has shown reduced penetrance (Fig. 1D).

The other sequence variation, c.1078C ⋙ T, created a premature termination codon p.Q360* in the exon 9 (Fig. 1A). It has been identified as a *de novo* mutation in Patient 2, a 12-years old girl with EMAS, who displayed motor disorders and mild neuropsychological impairments (Table 1). The lumbar puncture has shown low CSF/blood glucose ratio (0.38 mmol/l). The treatment with AEDs has given a partial effect only.

The aCGH analysis detected a 164.26 Kb microdeletion in the 1p34.2 chromosome region (Fig. 1E). It encompassed exons 2–10 of the *SLC2A1* gene and 10 additional adjacent genes, 4 of them (*C1orf50, CCDC23, ZNF691* and *ERMAP*) protein-coding. It appeared *de novo* in Patient 3, a 14-years old boy with a therapy-resistant GGE who was born with congenital cataract and microphtalmia after pregnancy complicated by viral infection. A psychomotor development delay has been observed.

4. Discussion

Using a combined approach of direct sequencing followed by aCGH, we have identified 3 different genetic defects that involve the *SLC2A1* gene. Preliminary data about CSF glucose levels were available in Patient 2 only. Although hypoglycorrachia is a valuable diagnostic marker for GLUT1-DS, patients tend to decline it because of the invasive procedure. It is a common decision in the mild clinical cases having good outcome, as it is in Patient 1. He has been selected for the *SLC2A1* mutation screening based on the EEG data for CAE/JAE. In the intricate clinical cases, like in Patient 3, the identification of the genetic defect is crucial for the differential diagnosis of GLUT1-DS and plays a key role for the successful treatment of the disease.

Patient 3 was initially suspected to have metabolic encephalopathy. The metabolic screening, however showed negative results. Mitochondrial disease has been excluded by muscle biopsy and mitochondrial DNA analysis. The diagnosis of GLUT1-DS was discussed after the oral glucose tolerance test (OGTT) showed a flat curve [7]. MLPA analysis was performed (data

Table 1
Clinical data of Bulgarian patients carrying SLC2A1 gene mutations.

Patient	1	2	3
Mutation	c.480C ⋙ A/p.H160Q	c.1078C ⋙ T/p.Q360X	1p34.2 loss
CSF/Blood glucose	Not available	1.82 mmol/l/4.73 mmol/l	1 Not available
Inborn defects	Not present	Not present	Bilateral cataract, Microphthalmia
Epilepsy syndrome	IGE (CAE or JAE)	EMAS	GGE
Age at Onset/ Examination/ Follow-up	10y/10y/13y	2y/12y	3 m/10y/12y
Type of seizures	GTCS	GTCS, atonic, myoclonic seizures	GTCS, myoclonic-atonic seizures, absences
Seizure Frequency/ Duration	1 GTCS/2–3 min	1–3 times daily GTCS – up to 10 min	2–3 per year GTCS/1–5 min. Multiple myoclonic-astatic seizures and absences starting at 3y 6 m
Motor disorder	Not present	Ataxia, Intention tremor, Titubation Mild hypotonia	Cerebral palcy, Quadri-pyramidal and Cerebellar syndrome
Neuropsycological development	normal	Learning disabilities, Mild Mental retardation, Depression	Moderate Mental retardation
Extraneurological features	Not present	Not present	Tachycardia, Hypercholesterolemia Impaired glucose tolerance[a] Obesity, Gynecomastia
EEG data at onset	Generalized paroxysmal activity of spikes-waves complexes 3 Hz, spontaneously and during hyperventilation, typical for absences, without certain clinical manifestation	Left frontal paroxysmal activity with synchronization to the right	Migrating focal discharges
EEG Follow-up	Normal EEG activity after VPA initiation	na	Generalized paroxysmal activity of spikes/ poly spikes waves complexes 3 Hz
NMR	Normal	na	Bilateral temporal dysplasia
Treatment	Valproate	Valproate + Topiramate	Valproate, Clonazepam, Lamotrigine, Nitrazepam, Levetiracetam in different combinations
Outcome	Seizure-free	Rare atonic seizures	Rare GTCS

[a] Flat curve of oral glucose tolerance test.

not reported) which had not given indication for a deletion in the *SLC2A1* gene. The aCGH analysis was proposed based on the complex clinical phenotype of intractable epilepsy, developmental delay and extraneurological features.

The overall frequency of the *SLC2A1*-mutations amongst Bulgarian patients with GGE (8.1%) is in the range that has been reported in the previous studies (1–10%) [4,5]. They have been found in clinical cases that differ notably by their severity. Patient 1 carrying missense mutation developed mild epilepsy phenotype and responded well to the treatment with AED, while in the patients with *SLC2A1*-truncation/deletion the clinical picture included all three neurological consequences characteristic for GLUT1-DS. We have not identified *SLC2A1*-mutations in patients with EOAE that have been widely discussed previously showing up to 12% frequency in that group [4].

The identified sequence variations exhibited different effects on the GLUT1 protein structure and function. The missense mutation p.H160Q is localized in one of the amphipathic α-helices lining the glucose permeation pathway [8,9]. The affected amino acid seems to be functionally important since its replacement with cysteine has resulted in a moderate reduction of the GLUT1 glucose transport activity [8]. Previous simulation studies suggested that p. H160 formed a hydrogen bond with the glucose molecule and that interaction had been compliant with a specific distance between the reacting groups [10]. Our 3D-analysis showed the possibility that the mutant amino acid p.Q160 did not fit into the correct position to satisfy that requirement (Fig. 1D).

The nonsense mutation virtually eliminated the last three transmembrane and the cytoplasmic domains of the GLUT1-sequence (Fig. 1C). Such *SLC2A1*-mutations lead to a protein premature degradation and lower the GLUT1-concentration to 50% resembling the pathologic effect of hemizygous mutations [11]. Patient 3 carrying 1p34.2 microdeletion showed more severe epilepsy phenotype, developmental delay, extraneurological features, and inborn defects that could not be attributed to the four other protein-coding genes in the deleted region. The clinical picture in this patient most closely resembles the “classic” GLUT1-DS phenotype.

Our study enriches the mutation spectrum of the *SLC2A1* gene by 3 novel cases that reflect the genetic and phenotypic diversity of GLUT1-DS and brings new insights in the molecular pathology of that disorder. The clinical data showed that the *SLC2A1* genetic defects should be considered equally in the entire range of the clinical manifestations of GGE paying attention to the extraneurological features. The aCGH analysis should be considered as an ultimate step during the diagnostic procedure of GLUT1-DS in patients with a complex clinical picture of intractable epilepsy involving neuropsychological impairments and accompanied by extraneurological features.

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Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.seizure.2017.11.014.

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