JIMD Reports
DOI 10.1007/8904_2013_253

RESEARCH REPORT

No Mutation in the *SLC2A3* Gene in Cohorts of GLUT1 Deficiency Syndrome–Like Patients Negative for *SLC2A1* and in Patients with AHC Negative for *ATP1A3*

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Received: 11 June 2013 / Revised: 4 July 2013 / Accepted: 5 July 2013 / Published online: 4 September 2013

Abstract The facilitative glucose transporter-1 (GLUT1) deficiency or de Vivo syndrome is a rare neuropediatric disorder characterized by drug-resistant epilepsy, acquired microcephaly, delayed psychomotor development, intermittent ataxia, and other paroxysmal neurological disorders due to the presence of dominant mutations in the *SLC2A1* gene. Alternating hemiplegia of childhood (AHC) is another rare neuropediatric disorder characterized by episodes of hemiplegia developing during the first 1.5 years of life. Before the recent finding of the gene *ATP1A3* as the major cause of AHC, a heterozygous missense mutation in the *SLC2A1* gene encoding GLUT1 was described in one child with atypical AHC, suggesting some clinical overlap between AHC and GLUT1 deficiency syndrome (GLUT1DS1). Half of patients with symptoms evocative of GLUT1DS1 with hypoglycorrhachia and up to 25 % of patients with AHC remain molecularly undiagnosed. We investigated whether mutations in *SLC2A3* encoding GLUT3, another glucose transporter predominant in the neuronal cell, may account the case of a cohort of 75 *SLC2A1* negative GLUTDS1-like patients and seven patients with AHC who were negative for *ATP1A3* and *SLC2A1* mutations. Automated Sanger sequencing and qPCR analyses failed to detect any mutation of *SLC2A3* in the patients analyzed, excluding this gene as frequently mutated in patients with GLUT1DS1 like or AHC.

Communicated by: Daniela Karall

Competing interests: None declared

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Abbreviations

AHC	Alternating hemiplegia of childhood
CSF	Cerebrospinal fluid
ENRAH	European Network for Research on Alternating Hemiplegia
GLUT1	Glucose transporter-1
GLUT1DS1	GLUT1 deficiency syndrome-1
GLUT1DS2	GLUT1 deficiency syndrome-2
PED	Paroxysmal exercise-induced dyskinesia

Introduction

Glucose transporter protein 1 (GLUT1) deficiency or de Vivo syndrome (GLUT1DS1 OMIM#606777) is a rare severe neurological disease with hypoglycorrhachia due to impaired

glucose transport across the blood–brain barrier of capillary endothelial cells (Leen et al. 2010). This syndrome classically presents with encephalopathy with drug-resistant epilepsy, acquired microcephaly, delayed psychomotor development, intermittent ataxia, and other paroxysmal neurological disorders. Abnormal movements, such as chorea of the extremities or paroxysmal dyskinesia induced by exercise, are sometimes observed in isolation without epilepsy (PED) (GLUT1DS2: OMIM#612126). Dominant mutations in *SLC2A1* encoding GLUT1 are found in only 20 % of patients with symptoms evocative of GLUT1 deficiency, suggesting the implication of other genes(Klepper and Leiendecker 2007).

Alternating hemiplegia of childhood (AHC, OMIM#104290) is another rare neurodevelopmental disorder. Six clinical criteria are to be considered for the diagnosis of AHC and include: (1) onset of paroxysmal events before 18 months of age; (2) repeated attacks of hemiplegia lasting from a few minutes to several days and involving either side of the body; (3) episodes of bilateral hemiplegia or quadriplegia; (4) other paroxysmal disturbances occurring during hemiplegic bouts or in isolation; (5) immediate disappearance of symptoms upon sleep; and (6) developmental delay and nonparoxysmal neurologic abnormalities such as dystonia, ataxia, cognitive impairments, and epileptic events in up to 50 % of patients (Panagiotakaki et al. 2010).

Dominant mutations in *ATP1A3*, encoding the Na+/K+−ATPase α3 subunit, recently emerged as the major cause for AHC since they account for more than 74 % of AHC cases according to studies (Heinzen et al. 2012; Rosewich et al. 2012). Moreover, dominant mutations in the *SLC1A3*, *CACNA1A*, and *ATP1A2* genes, respectively, encoding the glutamate transporter EAAT1, the α1 pore-forming subunit of the calcium channel Cav2.1, and the α2 subunit of the Na^+/K^+ ATPase pump, were first found as causing atypical cases of AHC (Jen et al. 2005; de Vries et al. 2008; Bassi et al. 2004). Interestingly, a *SLC2A1* mutation was found in one child fulfilling all criteria for AHC diagnosis except delayed age at onset of symptoms, hypoglycorrhachia, and deceleration of head growth not usually found in AHC but clinical criteria for GLUT1 deficiency (Rotstein et al. 2009). This case suggests some clinical overlap between AHC and GLUT1 deficiency syndrome. However, *SLC2A1* mutations were later excluded in a large cohort fulfilling all the criteria for AHC diagnosis, including seven patients studied here without mutations later found in *ATP1A3* (Vuillaumier-Barrot et al. 2011).

GLUT3 (encoded by the *SLC2A3* gene) is the predominant glucose transporter expressed by neuronal cells and has not yet been associated with disease in humans. *SLC2A3* and *SLC2A1* are not equally distributed in brain: *SLC2A1* is expressed in glial cells and *SLC2A3* in neuronal cells. Homozygous GLUT3-null mice are embryonic lethal, whereas heterozygous (GLUT3 +/−) mice display epilepsy and autistic behavior with stereotypies (Zhao et al. 2010). These observations suggest that GLUT3 may be associated with a dominant neurological disease in humans. In this report, we questioned the occurrence of *SLC2A3* mutations in *SLC2A1* negative GLUTDS1-like patients with epilepsy and mental retardation with or without hypoglycorrhachia, and in patients with typical AHC without mutation in *ATP1A3* or *SLC2A1*.

Methods

Patients

Eighty-two patients with a neurodevelopmental disorder molecularly undiagnosed were studied: 75 GLUTDS1-like patients with epilepsy who were negative for *SLC2A1* and seven patients with typical AHC and no mutations in the *ATP1A3* and *SLC2A1* genes. AHC patients, all previously described (Vuillaumier-Barrot et al. 2011), were included in the European Network for Research on Alternating Hemiplegia Registry (ENRAH) from which clinical data were extracted (Panagiotakaki et al. 2010). Collections were undertaken with the informed consent of patients and their legal representative in accordance with the relevant bioethics legislation.

Table 1 summarizes the clinical data of the 75 GLUT1DS1-like patients. All the patients presented with epilepsy (pharmacoresistant in 16 patients, and early onset epilepsy before 4 years for at least 44 patients), associated with abnormal movements in 15 patients and behavioral autism-like symptoms in seven patients. Four familial cases (dominant inheritance) were studied. The average age at the request of GLUT1DS1 molecular diagnosis was 7 years (1 month to 29 years). The ratio of cerebrospinal fluid (CSF)/plasma glucose concentrations was documented for 36 patients and was reported to be <0.5 for 16 patients (44 %) and >0.5 for 20 patients (56 %). A positive ketogenic diet response was observed for four patients.

The seven AHC patients tested were clinically representative of the French cohort of AHC and fulfilled all the criteria for AHC diagnosis (Vuillaumier-Barrot et al. 2011). All had a paroxysmal event before the age of 18 months. Two suffered from at least one epileptic seizure (Table 2).

Molecular Study

Genomic DNA was isolated from blood samples using standard phenol-chloroform procedures or Qiacube kit (Qiagen, Valencia, CA). *SLC2A3* was analyzed by automated

Table 1 Clinical description of the 75 GLUT1DS1-like patients

Clinical and biological data	Number of patients
Sporadic/familial	71/4
H/F	41/32
Age	1 month to 29 years (mean 7 years)
Microcephaly	5 (7 %)
Epilepsy (absences or myoclonies)	75 (100 %)
Pharmacoresistance	16 (21 %)
Early onset epilepsy (before 4 years)	44 (59 %)
Ketogenic diet positive response	4 (5 %)
Ataxia	6 (8 %)
Paroxystic dyskinesia	11 (15 %)
Autistic behavior	7 (9 %)
CSF/blood glucose <0.5 ($n = 36$)	16 (44 %)
CSF/blood glucose >0.5 ($n = 36$)	20 (56 %)

Table 2 Clinical description of the seven patients with AHC

Clinical and biological data at last examination	Number of patients
H/F	3/4
Age at last examination in years (mean)	7.5–52 (19.4)
Age at first paroxystic events in months (mean)	1–14 (4.2)
Age at first plegic attack in months (mean)	3–36 (9.8)
Repeated bouts of hemiplegia	7 (100 %)
Episodes of bilateral hemiplegia	5/5 (100 %, 2 unknown)
Abnormal eye movements	7 (100 %)
Disappearance upon sleep	6/6 (100 %)
Dystonia	6 (86 %)
Ataxia	7 (100 %)
Dysarthria	6 (86 %)
Seizures	0 (0 %)
Developmental delay	7 (100 %)
Autonomic dysfunction	7 (100 %)
CSF/blood glucose	Normal in 4 cases (not tested in other cases)

Sanger sequencing using the BigDye v3.1 reaction mix and the ABI 3130 capillary sequencer after polymerase chain reaction (PCR) amplification of the 10 *SCL2A3* exons and intron–exon boundaries using AmpliTaq Gold (Applied Biosystems, Carlsbad, California). Large deletions were searched in 35 patients without heterozygous SNPs using qPCR on two exons (exon 2 and 9). The GenBank [NM_006931] sequence was used as the reference. SNP data were obtained from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and frequency from Caucasian population-based databases (HapMap CEU population/CEPH). All primer sequences and PCR conditions are available upon request. Pathogenicity of unknown missense variant was assessed using five prediction software programs: Polyphen (http://genetics.bwh.harvard.edu/pph/), Panther (http://www.pantherdb.org/tools/csnpScoreForm.jsp), SIFT2 (http://blocks.fhcrc.org/sift/SIFT.html), SNPs3D (http://www.snps3d.org/), and Align GVGD (http://agvgd.iarc.fr/agvgd_input.php).

Results

Sequencing the 10 exons of *SLC2A3* in the 82 patients revealed five variants known as polymorphisms and two unknown variants in the heterozygous state: c.163T>A (p.Ser55Thr) in exon 3 in one GLUTDS1-like patient and c.966 +100T>C in intron 7 in one patient with AHC (Table 3). This intronic variant was found to be inherited by the unaffected mother, which excluded its pathogenicity. The Ser55 residue is not conserved between species. The p.Ser55Thr variant is predicted as nonpathogenic by four prediction softwares (Polyphen, SIFTV2, Panther, and SNPs3D) out of the five used. Align GVGD was the only one to predict that this variant could most likely interfere with function (class C55). Unfortunately, parent samples were not available to determine whether this variant was inherited.

Fifty-six patients were heterozygous for known SNPs. Twenty-six patients had no heterozygous SNPs and therefore might have a gene deletion in the heterozygous state resulting in haploinsufficiency. We therefore performed qPCR on two exons of the gene (exon 2 and 9) to determine whether their homozygous status was due to large-scale deletion. None of the 26 samples were found to harbor any copy number variation.

Discussion

Clinical and metabolic arguments led us to search mutations in GLUT3 in patients with epilepsy, mental retardation, or AHC. The analysis of 82 patients excluded *SLC2A3,* encoding GLUT3, as a major mutated gene in GLUT1DS1 and AHC.

The classical presentation of GLUT1DS1 includes intractable epilepsy developing in infancy with delayed development, ataxia, dystonia, and low CSF but normal serum glucose (hypoglycorrhachia). All types of *SLC2A1* mutations have been reported in GLUT1DS1, including hemizygosity due to

Table 3 Variants identified in the 82 patients (75 GLUT1DS1-like patients and 7 AHC) screened for *SLC2A3* mutation

Gene location	Sequence change	Protein change	SNP ID (dbSNP)	Number of heterozygous alleles/164	Number of homozygous alleles/164	Number of total alleles/164	Observed frequency of minor allele	Known frequency of HapMap CEU population
Exon 3	c.163T>A	p.Ser55Thr	Unknown	1	0	1	0.6 %	Unreported
Intron 3	c.269+36A>G	?	rs2541279	27	3	33	20.1 %	27 %
Exon 6	c.774A>G	p.Leu258=	rs17847967	18	5	28	17.1 %	15.8 %
Intron 7	c.966+100T>C	?	Unknown	1	0	1	0.6 %	Unreported
Intron 8	c.1069-65T>C	?	rs741361	39	7	53	32.3 %	36 %
Intron 9	c.1273 +131G>A	?	rs9668489	21	60	141	17.1 %	10.4 %
Exon 10	c.1308C>T	p.Thr436=	rs25684	38	20	78	47.5 %	40 %

large-scale genomic deletions (Leen et al. 2010). In our laboratory, the molecular screening of *SLC2A1*, including sequencing all coding exons and intron–exon junction, and searching for large deletion by MLPA identified a mutation in only 22 % of patients with GLUT1DS1-like presentation. Moreover, half of the GLUT1DS1-like patients with documented hypoglycorrhachia from our series had *SLC2A1* mutation, a result similar to Leen et al. who found *SLC2A1* mutations in 41 % of their 132 requests for *SLC2A1* analysis (Leen et al. 2010). These negative *SLC2A1* cases could be due to another presently unknown genetic defect, reversible transient glucose transport defect (Klepper et al. 2003), or even other nonidentified causes such as infectious, traumatic, some antiepileptic drugs (phenobarbital, valproate sodium). Elevated blood glucose level due to stress hyperglycemia, tested after lumbar puncture, may also result in lowered CSF/blood glucose ratio and so, to misdiagnosis. Alternatively, some *SLC2A1* mutated patients have been described with normoglycorrhachia, rendering this criteria not absolute for GLUT1-DS diagnosis (Mullen et al. 2010; Suls et al. 2008). One case of atypical AHC has been associated with a missense mutation in *SLC2A1*, which suggests clinical overlaps between the two pathologies (Vuillaumier-Barrot et al. 2011). The seven AHC patients included in our analysis were typical ones, fulfilling all criteria for typical AHC diagnosis but did not display *ATP1A3* mutation, the major cause of typical AHC. We hypothesized that *SLC2A3* could be a candidate in some of these AHC patients as for GLUT1DS1-like patients with or without hypoglycorrhachia. The commonly accepted "astrocyte-neuron shuttle hypothesis" (Pellerin and Magistretti 2011) that describes energy metabolism at the cellular level in the brain would predict that patients with *SLC2A3* mutations may suffer from normal to high CSF glucose levels rather than hypoglycorrhachia as for *SLC2A1* mutated patients. Autistic features are not a characteristic of GLUT1DS or AHC, which shows on the contrary a happy outgoing behavior, but it was suspected to be a feature of human GLUT3 deficiency as heterozygous GLUT3-null mice (GLUT3 +/−) display epilepsy and autistic behavior with stereotypies. In our GLUT1DS1-like cohort, seven patients on 75 had behavioral autism-like disorders.

Therefore, we studied *SLC2A3* and sequencing the 10 exons of *SLC2A3* did not reveal any obvious changes except five variants known as polymorphisms and two unknown benign variants in the heterozygous state. The observed frequency of known polymorphisms was close to that observed in the Caucasian population-based databases (HapMap CEU population/CEPH). One unknown missense variation (p.Ser55Thr) was observed in the heterozygous state in one patient with GLUT1DS1-like phenotype. Serine 55 is neither conserved between species nor among the class I GLUT transporters (GLUT1 to GLUT4), and p.Ser55Thr

was predicted as nonpathogenic by four prediction softwares, which arguments for its benign status. Heterozygous large gene deletion was also excluded, either by observation of heterozygosity or quantitative PCR.

Taken together, our results indicate that *SLC2A3* is not a major gene accounting for GLUT1DS1-like phenotype without *SLC2A1* mutations or typical AHC negative for *ATP1A3* and *SLC2A1*. The absence of known human pathology associated with GLUT3 could be related to the presence of GLUT6 and GLUT8 glucose transporters in neurons (that may supplement GLUT3). This is also consistent with the fact that GLUT3 haploinsufficiency in mice do not display any decrease in brain glucose utilization as determined by fluorodeoxyglucose micro-PET (Stuart et al. 2011). Furthermore, it is commonly reported that glucose utilization is more important in astrocytes than in neurons (Bouzier-Sore et al. 2006). An in vivo study has even shown that the brain prefers lactate over glucose as an energy substrate when both substrates are available (Wyss et al. 2011).

Candidate genes involved in other parts of cerebral energetic metabolism could be considered at least for GLUT1DS1-like, such as genes encoding molecules that modulate the expression of GLUT1: transcription factors, genes involved in posttranslational modifications like glycosylation or phosphorylation and other energy substrate transporters. An actual promising way to find new mutated genes is the high throughput sequencing approach, either exome or whole genome sequencing. Both AHC and GLUT1 deficiency syndromes occur de novo, which will facilitate the search for new genes in our cohorts by performing trio analyses, that is, comparing the sequence of the index case to the sequence of the two parents.

Acknowledgments We thank all the participating families and physicians. We thank the ENRAH for SMEs consortium supported by grant (LSSM-CT-2005-516513 ENRAH for SMEs) of the European Commission Research Programme FP6, especially its validation committee, for the clinical validation on the patients with AHC. This work was funded by "*La fondation Jerome Lejeune*", *l'association française contre les myopathies* (AFM), and "*l'association française de l'hémiplégie alternante de l'enfant"* (AFHA).

Competing Interest

None declared

Synopsis

No mutation found in the *SLC2A3* gene in cohorts of 75 GLUT1 deficiency syndrome–like patients negative for *SLC2A1* and in seven patients with AHC negative for *ATP1A3* and *SLC2A1*.

Conflict of Interest

C Le Bizec, S Nicole, E Panagiotakaki, N Seta, and S Vuillaumier-Barrot declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975, as revised in 2000.

Informed consent was obtained from all patients for being included in the study.

Details of the Contributions of Individual Authors

C Le Bizec did the work (technical results). S Nicole, E Panagiotakaki, N Seta, and S Vuillaumier-Barrot planned, conducted, and reported the work.

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