Glucose metabolism transporters and epilepsy: only GLUT1 has an established role

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BRIEF COMMUNICATION

Glucose metabolism transporters and epilepsy: Only GLUT1 has an established role

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Summary

The availability of glucose, and its glycolytic product lactate, for cerebral energy metabolism is regulated by specific brain transporters. Inadequate energy delivery leads to neurologic impairment. Haploinsufficiency of the glucose transporter GLUT1 causes a characteristic early onset encephalopathy, and has recently emerged as an important cause of a variety of childhood or later-onset generalized epilepsies and paroxysmal exercise-induced dyskinesia. We explored whether mutations in the genes encoding the other major glucose (GLUT3) or lactate (MCT1/2/3/4) transporters involved in cerebral energy metabolism also cause generalized epilepsies. A cohort of 119 cases with myoclonic astatic epilepsy or early onset absence epilepsy was screened for nucleotide variants in these five candidate genes. No epilepsy-causing mutations were identified, indicating that of the major energetic fuel transporters in the brain, only GLUT1 is clearly associated with generalized epilepsy.

KEY WORDS: Glucose transporter, Lactate transporter, Glucose metabolism, Generalized epilepsy, GLUT1 deficiency.

The glucose transporter GLUT1 plays an essential role in brain metabolism by facilitating transport of glucose in the neurovascular unit. Mutations in the *SLC2A1* gene that encodes GLUT1 lead to deficiency of the transporter and an expanding spectrum of neurologic disorders. Originally described as a cause of a severe encephalopathy,[1] GLUT1 deficiency has recently been shown to account for 10% of patients with early onset absence epilepsy (EOAE),[2,3] 5% of those with myoclonic astatic epilepsy (MAE),[4] and 1% of all individuals with genetic generalized epilepsies (GGEs).[5] In addition to seizures, subjects with more severe phenotypes may have developmental delay or ataxia. Others may have paroxysmal exercise-induced dyskinesia (PED) with or without epilepsy. An early diagnosis of GLUT1 deficiency is important as it is managed with the ketogenic diet, which typically results in seizure control and may improve cognition.

Because glucose metabolism is critical for neural activity, we hypothesized that mutation of other critical transporters in the cerebral energy metabolism pathways (Fig. 1) may also cause generalized epilepsies. In phenotypes known to have a significant association with GLUT1 deficiency we used Sanger sequencing to screen the other major cerebral glucose (GLUT3) and lactate (MCT1/2/3/4) transporters.

Materials and Methods

Clinical phenotyping

We selected 119 patients with the two generalized epilepsy syndromes most frequently associated with GLUT1 deficiency—myoclonic astatic epilepsy (n = 80)

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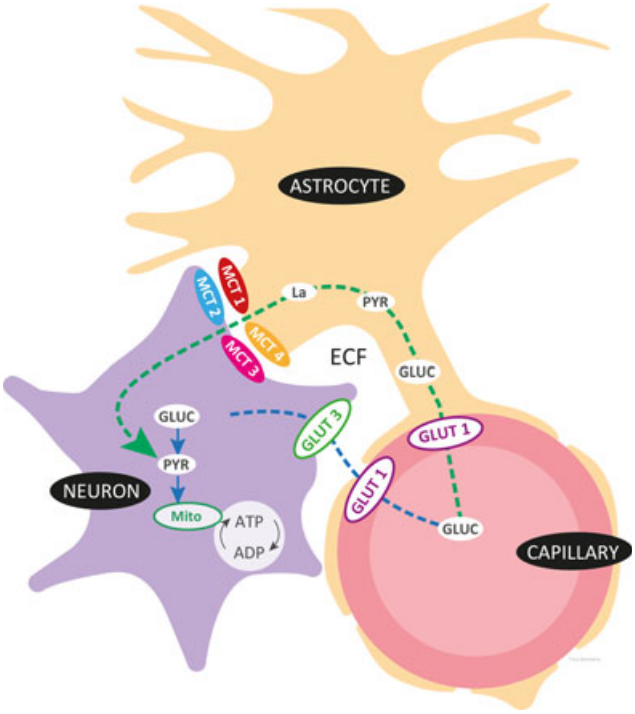

Figure 1.
Glucose and lactate transport pathways. Graphic showing a simplified version of the major glucose and lactate transport pathways in the brain. The key transfer steps facilitated by the GLUT and MCT transporters are illustrated. Gluc, glucose; Pyr, pyruvate; La, lactate; Glu, glutamate; ECF, extracellular fluid. Adapted from.[9]
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and early onset absence epilepsy (n = 39)—from the Epilepsy Research Centre database. In all patients, nucleotide and copy number variations in *SLC2A1* (GLUT1) had been excluded as part of previous studies.[2–4] The phenotype of MAE was specifically selected, as this disorder is regarded as the epilepsy syndrome most responsive to the ketogenic diet.[4] Myoclonic-astatic epilepsy was defined by onset of multiple afebrile, generalized seizure types between 6 months and 6 years, generalized spike-wave (>2.5 Hz) on electroencephalography (EEG), and at least one of myoclonic, atonic, or myoclonic-atonic seizures. Patients with tonic seizures were excluded. This is in line with our previously published work on GLUT1 deficiency[4] and based on the definition put forward by Oguni and colleagues.[6] Early onset absence epilepsy was defined by onset of typical absence seizures before age 4 years as the major seizure type and generalized spike-wave (>2.5 Hz) on EEG. Those with atonic, myoclonic-atonic, or tonic seizures were excluded from EOAE.[3] Detailed electroclinical information was obtained regarding the epileptology, developmental course, and medical and family history.[2,4] Genomic DNA was extracted from venous blood by standard methods.

The Austin Health Human Research Ethics Committee, Melbourne, Australia, approved this study (Project No. H2007/02961). Informed consent was obtained from all subjects, or their parents and carers in the case of minors or those with intellectual disability.

Polymerase chain reaction and Sanger sequencing

Five candidate genes of the solute carrier (SLC) transporter family—*SLC2A3* (GLUT3), *SLC16A1* (MCT1), *SLC16A7* (MCT2), *SLC16A8* (MCT3), and *SLC16A3* (MCT4)—encoding all major brain glucose or lactate transporters except GLUT1 (Fig. 1) were amplified using gene-specific primers designed to the reference human gene transcripts (NCBI Gene; http://www.ncbi.nlm.nih.gov/). Primer sequences are available on request. Amplification reactions were cycled using a standard protocol on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA, U.S.A.). Bidirectional sequencing of all exons and flanking regions including splice sites was completed with a BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. Sequencing products were resolved using a 3730xl DNA Analyzer (Applied Biosystems). All sequencing chromatograms were compared to published complementary DNA (cDNA) sequence; nucleotide changes were detected using Codon Code Aligner (CodonCode Corporation, Dedham, MA, U.S.A.).

Results

Clinical syndromes

The EOAE cohort had a median onset age of 18 months and, of those with sufficient data, 22 (63%) of 35 were refractory to trials of two appropriate medicines in adequate doses. The MAE cohort was more refractory, with 56 (80%) of the 70 with sufficient data showing no response to two medications. Around 60% (46/75) of MAE cases had an encephalopathic course, with a poor intellectual outcome following initial regression not reversed by treatment. Of the MAE cases, 19 (24%) of 80 received the ketogenic diet, mainly those diagnosed in the last 10 years when this treatment was more easily available. The majority of those with follow-up data showed at least a 50% seizure response to the diet (11/16, 69%), whereas two (2/16, 13%) had complete remission with the diet.

Candidate gene screening

Screening of *SLC2A3* revealed three heterozygous coding substitutions; a novel missense change, p.Ile14Leu, that is likely to be benign because isoleucine and leucine are functionally equivalent amino acids, and two known coding polymorphisms (p.Arg91His and p.Leu258Leu; Table 1). In *SLC16A1* we identified only one known heterozygous coding polymorphism, p.Arg490Glu, whereas in *SLC16A7* we detected four heterozygous coding polymorphisms, none

Table 1. Gene variants identified in our patient cohort

Gene	Protein	Location	Type	DNA level	Protein level	SNP ID[a]
SLC2A3	GLUT3	Coding	Missense	c.40A > C	p.Ile14leu	–
SLC2A3	GLUT3	Coding	Missense	c.272G > A	p.Arg91His	rs145936296
SLC2A3	GLUT3	Coding	Synonymous	c.774A > G	p.Leu258Leu	rs17847967
SLC16A1	MCT1	Coding	Missense	c.1470T > A	p.Asp490Glu	rs1049434
SLC16A7	MCT2	Coding	Synonymous	c.334C > T	p.Leu112Leu	rs200049323
SLC16A7	MCT2	Coding	Missense	c.656G > T	p.Ser219Ile	rs142586562
SLC16A7	MCT2	Coding	Missense	c.1333A > T	p.Thr445Ser	rs3763980
SLC16A7	MCT2	Coding	Synonymous	c.1383C > T	p.Asn461Asn	rs3763979
SLC16A8	MCT3	Noncoding	5′UTR	c.-5C > T	–	–
SLC16A8	MCT3	Coding	Missense	c.124T > C	p.Phe42Leu	–
SLC16A8	MCT3	Noncoding	Splicing	c.214+1G > C	–	rs77968014
SLC16A8	MCT3	Coding	Missense	c.1384G > T	p.Asp462Tyr	rs144999316
SLC16A8	MCT3	Coding	Missense	c.1450G > A	p.Ala484Thr	–
SLC16A3	MCT4	Coding	Missense	c.883G > A	p.Val295Ile	[b]
SLC16A3	MCT4	Coding	Synonymous	c.831G > A	p.Ala277Ala	–
SLC16A3	MCT4	Coding	Synonymous	c.1362C > T	p.Asn454Asn	rs112638041

[a]Single nucleotide polymorphism identification (rs) number.
[b]Present at low frequency on Exome Variant Server database but not assigned an rs number.

of which appear to be disease-causing based on their frequency in control populations (Table 1).

We detected most variation in the *SLC16A8* gene with five heterozygous variants found including two known polymorphisms and three novel changes (Table 1). Of the novel changes one was located in the 5′UTR (c.-5C > T) and two were missense changes (p.Phe42Leu and p.Ala484Thr). Parents were available and segregation analysis was completed for all five variants; however, all were found to be inherited from unaffected parents, making it unlikely that any are disease-causing.

In *SLC16A3,* three heterozygous variants were detected only one of which (p.Ala277Ala) was novel but synonymous. Although rare, the p.Val295Ile variant in *SLC16A3* is predicted to be benign by standard pathogenicity prediction software (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/) and the substitution has a low Grantham score of 29, suggesting that the variant is unlikely to be damaging to the MCT4 protein.

The *SLC2A3* and *SLC16A7* genes are predicted to be tolerant to functional change according to their residual variant intolerance scores (RVIS) of 0.619 and 0.375, respectively.[7] Although the *SLC16A1* (RVIS = −0.072) is close to expectation and the *SLC16A1* (RVIS = −0.641) gene is predicted to be intolerant to functional change, no RVIS was available for *SLC16A8*. These intolerance predictions correlate well with the number of variations identified for each gene (Table 1).

Discussion

The discovery of mutations in *SLC2A1* in a wide spectrum of mild to severe generalized epilepsies was unexpected but important because of treatment implications. However, our hypothesis that similar cases are due to mutations in other glucose and lactate transporters was not supported.

Our data suggest that GLUT1 is the only energy metabolism transporter associated with generalized epilepsy. This could be because it is the only glucose transporter present in the neurovascular unit and thus the rate-limiting step in brain glucose transport. However, GLUT1 is also expressed in astrocytes. Although other members of the GLUT family are expressed in the brain, only GLUT1 and GLUT3 are thought to contribute significantly to brain glucose transport (reviewed in[8]). The exclusive expression of GLUT3 in neurons makes it an excellent candidate for an epilepsy gene; however, we were unable to find GLUT3 mutations in the generalized epilepsy cohort screened here. Similarly, if energy delivery to neurons is dependent on the astrocyte-neuron lactate shuttle,[8] then the lactate transporters would also be attractive epilepsy gene candidates. Although no mutations were identified, it may be that these transporters are so crucial that any deficiency is compensated for by the presence of other known or as yet unidentified transporters. In some regions of the brain, multiple transporters are expressed, as shown in Fig. 1, providing the opportunity for redundancy. Such compensation has been observed in a mouse model of *Glut1* haploinsufficiency; developmental expression of *Mct1* and *Mct2* was increased twofold in the neonatal brain in response to reduced *Glut1* in heterozygous null mice.[9]

Studies from other animal models are broadly consistent with our findings. Null heterozygous *Glut3* mice exhibited behavioral features suggesting cognitive and social impairments as well as electrographic discharges but no clinical generalized seizures.[10] Of interest, haploinsufficiency of *Glut3* was associated with increased concentration of micro-

vascular/glial *Glut1* and *Mct2*, no change in brain glucose, and enhanced lactate uptake. Likewise, in an independent study, *Glut3* haploinsufficiency did not impair mouse brain glucose uptake or utilization.[11] In a study of monocarboxylate transporters targeted deletion of *Mct3* in mouse altered visual function; however, there were no overt seizures, although these were not specifically tested for.[12]

This study has some limitations. First, certain types of pathogenic variants that we could not detect by Sanger sequencing, such as large copy number variants (CNVs), may exist in these genes. However, this is unlikely, since most mutations identified in GLUT1 deficiency are single nucleotide variants that are readily detectable by our methods and often cause moderate to severe disease. Second, the infrequent inherited gene variants we found may reduce the amount of transporter, but on their own are insufficient to cause disease. Such variants could still behave as susceptibility variants. To test this would require functional studies and, as a common variant association study, a much larger sample size. Third, we intentionally screened the two generalized epilepsy syndromes most frequently associated with GLUT1 deficiency: MAE and EOAE. However, this does not exclude the involvement of the GLUT3 and MCT transporters in the pathogenesis of other types of epilepsy, such as temporal lobe epilepsy, where reduction in brain MCT1 has been reported.[13,14]

Thus the molecular explanation for syndromes that may respond to the ketogenic diet where GLUT1 variants are not found remains elusive. Analysis with hypothesis-free massively parallel sequencing may elucidate the molecular determinants in these patients. Our results are also consistent with the negative findings of an independent screen of GLUT3 in patients with GLUT1 deficiency syndrome-like disease published while we were writing this report.[15]

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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