Early-Onset Absence Epilepsy Caused by Mutations in the Glucose Transporter GLUT1

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Absence epilepsies of childhood are heterogeneous with most cases following complex inheritance. Those cases with onset before 4 years of age represent a poorly studied subset. We screened 34 patients with early-onset absence epilepsy for mutations in SLC2A1, the gene encoding the GLUT1 glucose transporter. Mutations leading to reduced protein function were found in 12% (4/34) of patients. Two mutations arose de novo, and two were familial. These findings suggest GLUT1 deficiency underlies a significant proportion of early-onset absence epilepsy, which has both genetic counseling and treatment implications because the ketogenic diet is effective in GLUT1 deficiency.


Absence epilepsies are an important problem in children. The classic syndrome is childhood absence epilepsy (CAE) where absence seizures typically begin between 4 and 10 years in an otherwise healthy child. 1,2 CAE is well recognized, although there is ongoing debate regarding the phenotypic limits and precise criteria for diagnosis. 2,3 For example, some would exclude children with generalized tonic-clonic seizures in addition to absence seizures. 2,3 Patients with early-onset absence seizures (before 4 years of age) are uncommon and may have more complex phenotypes with additional seizure types, movement disorders, or intellectual impairment. 4

A disorder apparently unrelated to CAE is encoding the glucose transporter type 1 (GLUT1) deficiency syndrome (GLUT1-DS) classically comprising infantile-onset seizures, complex movement disorders, ataxia, and intellectual disability with microcephaly in some children. 5 GLUT1-DS is due to heterozygous mutations in SLC2A1 encoding GLUT1, the molecule transporting glucose across the blood–brain barrier. 6 Hypoglycorrhachia, caused by impaired glucose transport, is a key diagnostic feature. 7 Generalized epileptiform spike-wave patterns are seen, and heterogeneous seizure types including absence, myoclonic, and tonic-clonic seizures have been reported, but an homogeneous epilepsy syndrome has not been recognized. 5 Treatment with the ketogenic diet is effective for seizure control. 7

More recently, a wider phenotypic spectrum associated with GLUT1 deficiency has been reported including paroxysmal exercise-induced dyskinesia, later onset seizures, often normal intellect, and even normal cerebrospinal fluid (CSF)/serum glucose ratios. 9–12 Critical review shows that absence seizures, particularly in younger children, emerge as a prominent seizure phenotype. 8–13 We hypothesized that GLUT1 deficiency may underlie a significant proportion of early-onset absence epilepsies.

Patients and Methods

Patients

Thirty-four patients with onset of absence epilepsy before 4 years of age and no evidence of a secondary cause for their epilepsy were included. This age cutoff is the widely accepted minimum onset age for CAE. 1 All had generalized spike-wave (>2.5 Hz) and absence seizures documented on electroencephalogram. Patients with atonic or tonic seizures were excluded. Informed consent was obtained from all patients and, for minors, their parents or legal guardians. Ethics approval for the study was provided by the Human Research Ethics Committee at the participating centers.

Mutation Analysis

Mutation analysis of all exons and intron-exon boundaries of SLC2A1 was performed on genomic DNA of patients and their parents, when available, by direct sequencing according to procedures described elsewhere. 12 Primer sequences can be obtained on request. To confirm the identified mutations in the patients and their absence in a control population of 276 ethnically matched subjects, we performed pyrosequencing on genomic DNA. Numbering of mutations started at A of the translation initiation codon, ATG, using complementary DNA sequence NM_006516.1.

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Potential conflict of interest: Nothing to report.

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Table. Clinical and Mutation Details of Early-Onset Absence Epilepsy Patients with GLUT1 Mutations

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Current Age</th>
<th>Age of Onset</th>
<th>Exon</th>
<th>Paroxysmal Dyskinesia</th>
<th>Seizure</th>
<th>Family History of Epilepsy</th>
<th>SLC2A1 Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F 28 yr 3 yr 7 yr</td>
<td>F</td>
<td>2 or 3</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Seizure free with VPA from 7 yr</td>
<td>Exon 5</td>
<td>c.660G&gt;C</td>
</tr>
<tr>
<td>2 M 28 yr 3 yr 8 yr</td>
<td>M</td>
<td>2 or 3</td>
<td>Mild gait ataxia</td>
<td>Normal</td>
<td>Normal</td>
<td>Mild ID (IQ, 56)</td>
<td>Daily absences on VPA + TPM</td>
<td>Exon 7</td>
</tr>
<tr>
<td>3 F 12 yr 14 mo</td>
<td>F</td>
<td>3 or 5</td>
<td>Mild gait ataxia</td>
<td>Normal</td>
<td>Subtle; 5 yr</td>
<td>Moderate ID (IQ, 48)</td>
<td>Sporadic absences on VPA + LTG</td>
<td>Exon 4</td>
</tr>
<tr>
<td>4 F 7 yr 13 mo 12 mo</td>
<td>F</td>
<td>3 or 5</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Frequent absences on VPA + LTG + ETX</td>
<td>Intron 5</td>
<td>c.680-11G&gt;A</td>
</tr>
</tbody>
</table>

DNA mutations were numbered using the reference sequence NM_006516.1. GTCS = generalized tonic-clonic seizure; VPA = valproic acid; TMD = transmembrane domain; ID = intellectual disability; IQ = intelligence quotient; TPM = topiramate; LTG = lamotrigine; ETX = ethosuximide.

RNA Isolation and Reverse Transcription Polymerase Chain Reaction
Total RNA of the patient with the intronic mutation, c.680-11G>A, and her mother was isolated from lymphoblast cell lines using the RNasy kit (Qiagen, Venlo, the Netherlands). After synthesis of first-strand complementary DNA with SuperScript III First-Strand Synthesis System for reverse transcription polymerase chain reaction (Invitrogen, Carlsbad, CA), we performed polymerase chain reaction amplification using primers annealing in SLC2A1 exons 4 and 8. Wild-type and aberrant polymerase chain reaction fragments were subsequently sequenced according to procedures described elsewhere.12

Functional Studies in Xenopus Oocytes
All experimental procedures have been described previously.11 In brief, the QuickChange kit (Stratagene, La Jolla, CA) was used to introduce mutations in the complementary DNA of SLC2A1 (pSP65 plasmid kindly provided by Dr Mike Mueckler13). The desired mutations were verified and other mutations excluded by sequencing all mutated clones. Plasmids were linearized and transcribed in vitro (Sp6 mMMessageMachine kit; Ambion, Austin, TX). Messenger RNA was injected into oocytes to study glucose uptake by zero-trans influx experiments with 3-O-methyl-D-glucose, and protein stability and surface expression using Western blots and immunocytochemistry (rabbit anti-GLUT1 antibody; Abcam, Cambridge, MA).

Results
Mutations were identified in 4 of 34 (12%) patients with early-onset absence epilepsy (Table). No clinical differences were observed between mutation-positive and -negative patients for age of onset, intellectual outcome, and electroclinical syndrome. On clinical review, after discovery of a SLC2A1 mutation, Patient 3 was found to have mild paroxysmal exercise-induced dyskinesia, previously considered an epileptic phenomenon. Subtle ataxia was found in Patients 2 and 3, possibly explained by high-dose antiepileptic drugs.

Three exonic missense mutations and one intronic splice-site mutation were identified (see the Table). Patients 1 and 2 inherited their missense mutations from a parent with idiopathic epilepsy, that is, juvenile absence epilepsy and adult-onset absence epilepsy. Examination of parental DNA of Patients 3 and 4 did not show the mutation, suggesting that the mutation in these children arose de novo (paternity was confirmed with a panel of 31 STR markers located on 15 different chromosomes). None of the 4 mutations was observed in 276 ethnically matched control subjects. In GLUT1-DS, different substitutions for R126 have been identified, including the cysteine substitution found here. Previous functional studies suggested that this substitution was pathogenic. R126 and S324 are highly conserved amino acids in different species. R223 is conserved only in mammals, but substitution of this polar by a nonpolar amino acid (proline), as found in our patient, is not observed in evolution (alignments in supplementary data).

Because the intronic mutation creates a new splice acceptor site, we assessed its effect on messenger RNA splicing using lymphoblast cell lines of the patient and her unaffected mother. An aberrant SLC2A1 transcript was demonstrated only in the patient. We observed an in-frame 9-base pair insertion, TCCCCCCAG, in front of exon 6, indicating that the intronic mutation results in the utilization of a cryptic splice acceptor site within intron 5. The mutation predicts the insertion of three amino acids PPV in the loop connecting transmembrane domains 6 and 7.
Functional Investigations of Mutant and Wild-Type GLUT1 Transporters

Glucose transport was decreased for all three GLUT1 missense mutations compared with wild type (Fig. A). All mutations markedly decreased the maximum transport velocity, $V_{\text{max}}$, without significantly affecting the Michaelis–Menten constant, $K_m$ (see Fig. B). There was no evident defect in production and stability of the mutant proteins (see Fig. C) or insertion in the cell-surface membrane (see Fig. D). Thus, all missense mutations reduced the transport capacity of GLUT1, probably without affecting glucose binding, protein stability, or intracellular transport mechanisms.

Discussion

SLC2A1 mutations were found in 12% of this cohort of 34 patients with early-onset absence epilepsy. The pathogenicity of these mutations is strongly suggested by the high conservation of the altered amino acids, the absence of these mutations in control subjects, the de novo occurrence of two of the mutations, and the in vitro studies showing a functional deficit of glucose transport for the three missense mutations. Because CSF measures are not routinely performed in milder generalized epilepsies, no CSF/serum glucose ratios were available for our patients. Also, it has recently been shown that, in patients with GLUT1 deficiency with milder phenotypes such as paroxysmal exercise-induced dyskinesia, the CSF/serum glucose ratio can be borderline or even normal.\textsuperscript{1,12}

The patients’ epilepsy was characterized by absence seizures as the predominant seizure type, onset before 4 years of age, and normal development before seizure onset.
The epilepsy varied from being easily controlled in some to refractory in others; intellect ranged from normal to moderately impaired. Thus, the seizure phenotype cannot be readily distinguished from CAE except for the earlier age of onset. Ataxia and dyskinesia, both mild, were diagnosed only in hindsight. Mild ataxia and intellectual disability were not exclusive to patients with \textit{SLC2A1} mutations (see Supplementary Table).

The ketogenic diet offers an alternative treatment because it is effective for seizures in classic GLUT1-DS. Although cognitive difficulties respond less well, improvement of intellectual abilities has been observed.\textsuperscript{7,11} Preliminary results of the ketogenic diet in Patients 3 and 4 showed a marked reduction of epileptiform activity on electroencephalogram.

Absence epilepsies usually follow complex inheritance where multiple genes are thought to contribute to the cause. The risk to first-degree relatives is about 8%.\textsuperscript{16} In contrast, the majority of patients with classic GLUT1-DS are isolated and the mutations arise de novo, meaning that the risk to siblings is negligible, as also found in two of four patients here with early-onset absence epilepsy. However, the milder, more recently recognized phenotypes with \textit{SLC2A1} mutations may be associated with autosomal dominant transmission with high penetrance. Thus, the risk to first-degree relatives approaches 50%, as observed in the other two patients. A molecular diagnosis of a \textit{SLC2A1} defect in a child with absence epilepsy, with subsequent investigation of the family, now allows accurate genetic counseling together with early diagnosis and treatment. Rarer identified molecular causes of early-onset absence epilepsy include mutations in a gene encoding a GABA receptor subunit (\textit{GABRG2}) and a sodium channel subunit gene (\textit{SCN1B}).\textsuperscript{17,18}

Molecular diagnosis for epilepsy has been a research tool until now with the exception of sodium channel mutations in Dravet syndrome where 70% to 80% of patients have mutations.\textsuperscript{19,20} Here we report the second gene with wide diagnostic utility for an idiopathic epilepsy syndrome. Therefore, we suggest that \textit{SLC2A1} mutational analysis is warranted in all children with absence seizures beginning before 4 years of age, enabling a molecular diagnosis with treatment and genetic counseling implications.

References

Acute basal ganglia necrosis in the pediatric age group is a neurological disorder characterized by symmetrical degeneration of the caudate nucleus, putamen, and occasionally the globus pallidus. It is typically preceded by an intercurrent febrile illness and is clinically manifested by truncal ataxia and hypotonia, transient limb paresis or fixed dystonic posturing, hyporeflexia, and a change in consciousness level.1,2 Sucking and swallowing are usually impaired but respiration is generally unaffected. The acute phase lasts days to weeks and in the sporadic patients, clinical recovery is often complete, though persistence of behavioral and movement symptoms and mild learning disabilities are sometimes seen.

The outcome is less favorable for the familial cases in which the course is relentlessly progressive or punctuated by recurrent episodes with involvement of additional brain regions and significant residual neurological damage. The differential diagnosis of the latter group includes mitochondrial respiratory chain defects mainly due to mutations in the ATP6 gene,3 organic acid disorders,4 Wilson disease,5 juvenile Huntington’s chorea,6 pantothenate kinase-associated neurodegeneration,7 biotin responsive basal ganglia disease,8 and the striatal necrosis associated with NUP62 mutation.9

We now report on the study of four patients who suffered from recurrent episodes of flaccid paralysis and encephalopathy associated with bilateral striatal necrosis.

Case Reports

An Illustrative Case

Patient II-1, 20 years old, is the oldest of five children born to consanguineous parents. Pregnancy, delivery, postnatal course, and early psychomotor development were all normal. At the age of 3.5 years, on the second day of a nonspecific upper respiratory infection, the patient became obtunded and weak over the course of few hours. On initial examination he was difficult to arouse, disoriented, and his speech was slurred. Proximal and distal muscle weakness was noted (biceps and shoulder abductor strength was 3/5). The patient could not rise and this was associated with decreased strength of the planter extensors and flexors (3/5 bilaterally). Deep tendon reflexes could not be elicited. Oral feeding was unsuccessful due to choking and nasogastric tube feeding was required. Over the course of 3 weeks, muscle strength and deep tendon reflexes returned to normal. Residual effects included difficulty with opening of tight jars and occasional falls attributed to minimal distal weakness. Cognitive functions returned to normal and there was no subsequent loss of milestones or developmental delay.

Thereafter, the patient remained in good health, started school at the appropriate age, and had no limitations in his daily activities. Beginning at 7 years of age, he was noted to have increasing difficulty with long distance walking, he fell frequently, complained of...