Imaging the Metabolic Footprint of Glut1 Deficiency on the Brain

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Cerebral 18F-fluorodeoxyglucose positron emission tomography in 14 patients with microcephaly, developmental delay, seizures, and mutations of the glucose transporter Glut1 (Glut1 deficiency syndrome) showed distinct abnormalities. Within a global context of diminished cortical uptake, more severe hypometabolism was found in the mesial temporal regions and thalami, accentuating a relative signal increase in the basal ganglia. In contrast, the structure of the brain appeared preserved in patients additionally investigated by magnetic resonance imaging. This metabolic footprint was relatively constant in all patients regardless of age, seizure history, or therapies and therefore constitutes a radiological signature of the disease. The full expression of the signature in the youngest patient (aged 19 months) indicates that the state of haploinsufficiency caused by Glut1 mutation leaves a permanent footprint on the nervous system from its earlier postnatal stages of development. The potential benefit of prompt diagnosis, aided by 18F-fluorodeoxyglucose positron emission tomography, and early initiation of available therapies is underscored by our results.

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Maintenance of constant global needs and meeting of task-induced regional energetic demands on the nervous system depends primarily on an abundant supply of oxygen and glucose. During postnatal development, the human brain acquires the ability to retain circulating glucose very efficiently as the different structures increase their metabolic rates to exceed those of the adult.1 Whether the increase in metabolism reflects the formation of relatively stereotyped age-related behaviors2 or whether it just fuels the exuberant cellular arborization and redundant synaptic connectivity that precedes the consolidation of the adult circuits3 remains to be clarified. Nevertheless, at approximately 10 years of life, the metabolic rate of the brain as a whole and of its individual components decreases to the level characteristic of the adult.2 It appears therefore that the nervous system will be most vulnerable to limited substrate availability when the demands are highest, that is, in infancy and early childhood, and that the constant insufficiency of glucose will impact the various cerebral structures in a time-dependent manner, as they are recruited into the hypermetabolic stage.

Circulating glucose enters the brain nearly exclusively by facilitated diffusion via the transporter Glut1.4 After diffusing through the endothelial cell, glucose gains access to the adjacent astrocyte, from which it may exit again toward the neuron. All of these steps are mediated by Glut1. The transporter is a membrane-spanning multifunctional glycoprotein that also may allow the passage of water and of dehydroascorbic acid among other small molecules.5–7 In the human brain, Glut1 appears very early during prenatal development and subsequently is subject to various forms of modulation or acquired deficiency,8,9 but it remains the sole glucose transporter of the cerebral endothelium and the primary one in astroglia. It appears therefore that a complete absence of Glut1 must be incompatible with life and that a partial deficiency will primarily cause cerebral dysfunction.

A human syndrome was described in 1991 by De Vivo and colleagues, in which the suspected (and later proved by genetic analysis) defect involved a primary deficiency of glucose transport into the brain.10 This entity was termed glucose transporter deficiency syndrome, or Glut1-DS. The condition manifests in infancy with acquired microcephaly, seizures resistant to anticonvulsants, intellectual and motor retardation, and ataxia. A constant feature is the presence of hypoglycorrachia, with a cerebrospinal fluid: blood glucose ratio of less than 0.33. To date, some 30 sporadic or familial mutations have been described in GLUT1, mapped to chromosome 1p.4,11,12 The result is, in the most unfavorable situation, a state of effective hemizygosity, al-
though several of the mutations simply cause dysfunction of an otherwise normally produced protein.

We set out to investigate cerebral glucose uptake by 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) in a series of 14 Glut1-DS patients carrying known mutations and afflicted by different disease severity. We expected to detect a decrease in uptake and found it to follow a well-defined regional pattern that appeared in early infancy and persisted into adult age.

**Patients and Methods**

**Patient Preparation**

Glut1-DS patients were identified based on several criteria: (1) clinical syndrome, (2) hypoglycorrhachia, (3) decreased erythrocyte glucose uptake (also mediated by Glut1), and (4) sequence analysis of the GLUT1 gene as previously described. Informed consent conforming to the guidelines of the Institutional Review Board of Columbia University and of the Columbia Kreitchman PET Center was obtained from patients and their parents. Patients fasted for at least 8 hours before the injection of the radiopharmaceutical. Only patients with a fasting glucose level less than or equal to 100mg/dl were included in the study. Intravenous access was obtained at least 15 minutes before the radiopharmaceutical administration. The patients then were injected with 0.14mCi/kg of 18F-2-deoxyglucose and scanned 30 minutes after injection.

**Medications Administered at Time of 2-Deoxy-2[18F] Fluoro-d-glucose Positron Emission Tomography**

Several patients (Table) were sedated just before scanning (approximately 25 minutes after isotope injection) using a maximum of 0.2mg/kg intravenous pentobarbital titrated to light sleep to prevent motion. One of these patients (identified as L in the Table) received several intravenous doses of midazolam (total dose 1.2mg/kg) given over an interval extending from immediately before isotope injection to the initiation of the scan, at which time a dose of pentobarbital (0.05mg/kg) was given. Cardiorespiratory function was monitored by pulse oxymetry and automated blood pressure, and heart rate determinations were made frequently before and during sedation and found to remain constant. All patients receiving sedation recovered full neurological performance within 5 hours after the termination of the scans.

**Data Acquisition, Processing, and Display**

Studies were acquired on a Siemens ECAT EXACT HR+ (Knoxville, TN) with resolution power = 4mm. Each study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Phenotype</th>
<th>Medications</th>
<th>Diet</th>
<th>Sedation</th>
<th>CSF/ Blood Glucose</th>
<th>Mutation</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>F</td>
<td>1.6</td>
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<td>None</td>
<td>Ketogenic</td>
<td>None</td>
<td>0.31</td>
<td>969 del c, c971t</td>
</tr>
<tr>
<td>B</td>
<td>F</td>
<td>6.1</td>
<td>Seizures age 3–24 months, spasticity, ataxia, hypotonia, hyporeflexia, normal EEG</td>
<td>None</td>
<td>Ketogenic</td>
<td>None</td>
<td>0.41</td>
<td>741 ins c</td>
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<td>C</td>
<td>M</td>
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<td>D</td>
<td>F</td>
<td>6.8</td>
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<td>Ketogenic</td>
<td>None</td>
<td>0.40</td>
<td>686 del ctc</td>
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<tr>
<td>E</td>
<td>F</td>
<td>7</td>
<td>Seizures since 3 mo, spasticity, hyperreflexia, slurred speech, abnormal EEG</td>
<td>L-carnitine, folate, multivitamins</td>
<td>Ketogenic, non-compliant</td>
<td>None</td>
<td>0.34</td>
<td>1086 del g</td>
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<td>F</td>
<td>F</td>
<td>9.3</td>
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<td>Regular</td>
<td>None</td>
<td>0.40</td>
<td>368 ins 23</td>
</tr>
<tr>
<td>G</td>
<td>F</td>
<td>10</td>
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<td>None</td>
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<td>T310I</td>
</tr>
<tr>
<td>H</td>
<td>M</td>
<td>10.3</td>
<td>Seizures since 1 mo, spasticity, ataxia, slurred speech, hyperreflexia, abnormal EEG</td>
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<td>0.35</td>
<td>266 del c, 2267c</td>
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<tr>
<td>I</td>
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<td>Valproate</td>
<td>Regular</td>
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<td>0.40</td>
<td>R335W</td>
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<td>0.31</td>
<td>E146K</td>
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<tr>
<td>L</td>
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<td>Incoordination, hyperreflexia, Babinski signs, speech slurring, terminal tremor, normal EEG</td>
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<td>0.39</td>
<td>R126H</td>
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<tr>
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<td>Epilepsy, familial transmission (son more affected), abnormal EEG</td>
<td>None</td>
<td>Regular</td>
<td>None</td>
<td>n.d.</td>
<td>R126C</td>
</tr>
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</table>

The age at the time of PET is indicated in years. Diet and medication dosages had been maintained constant for at least 6 mo before the study, and antiepileptic blood levels were found to conform to standard clinical reference ranges. The administration of sedation at the time of isotope injection is registered (see Patients and Methods). Cerebrospinal fluid to blood glucose ratios were obtained from samples drawn nearly simultaneously. Amino acid mutations are represented by (capital) native amino acid (in one-letter code), amino acid number, and mutant amino acid. Nucleotide mutations are indicated by nucleotide number, del for deletion or ins for insertion, nucleotide, or by native nucleotide, number, and mutant nucleotide.

FDG-PET = 18F-fluorodeoxyglucose positron emission tomography; CSF = cerebrospinal fluid; EEG = electroencephalogram; n.d. = not determined.
was acquired using a multiframe technique and auto attenuation correction in a dynamic scan mode (4 frames at 480sec/frame) that used filter back projection. Reconstruction with autoattenuation correction used a Hann filter (cutoff, 0.40 cycles/pixel). Postreconstruction transverse, oblique transverse, and coronal and sagittal plane images with a slice thickness of 0.50cm then were produced and displayed using an inverted gray scale and rainbow (16 step) maps.

**Image Interpretation and Analysis**

Images were interpreted qualitatively on transverse brain image file (slice thickness, 2.54mm). They were compared with a disease-free control by a nuclear neuroradiologist, who identified the main cerebral structures and evaluated uptake qualitatively.

**Additional Studies**

Selected patients received electroencephalograms within the 24 hours preceding the FDG-PET. Results were analyzed by an epileptologist. Magnetic resonance imaging (MRI) scans were also performed in a subgroup of patients (see Table). Using 1.5T MRI scanners, the following imaging sequences were obtained in the axial, coronal, and sagittal planes: T1 and T2 weighted, fluid-attenuated inversion recovery, and diffusion weighted. The images were interpreted by a neuroradiologist.

**Genetic Analysis**

DNA was extracted from patients’ blood after consent for genetic testing had been obtained according to standard methods described elsewhere. In brief, genomic DNA was purified and quantified, and samples were subjected to gel electrophoresis before polymerase chain reaction. Appropriate polymerase chain reaction primers were designed to yield DNA fragments spanning the entire GLUT1 coding region and intron-exon boundaries in chromosome 1. DNA was automatically sequenced, and mutations were confirmed by sequencing both strands. All patients used for this study carried one mutation in one allele.

**Results**

**18F-fluorodeoxyglucose Positron Emission Tomography of GLUT1 Subjects**

All images from all patients were satisfactory for study and conformed to the specifications expected from reagents and equipment. The most striking finding was a constant decrease in cortical uptake together with an unusual heterogeneity of the signal generated by the basal ganglia-thalamus complex. In particular, the thalamus displayed hypometabolism comparable to the degree of cortical depression, whereas the caudate and lentiform nuclei exhibited a relative increase in uptake. These findings were independent of the administration of sedatives and of the degree of severity of the disease, although detailed neuropsychological analysis is needed to confirm the latter postulate. Within the cerebral cortex, a more pronounced uptake deficit was observed in the mesial temporal lobes, which did not appear to correlate with performance impairment or coexistence of epilepsy.

**Structural Studies by Magnetic Resonance Imaging**

Several patients also were studied by cranial MRI 24 hours before the realization of PET (see Table). A subgroup of these received several additional MRI and computed tomography scans as part of past evaluations. All these results of brain imaging were normal, despite the microcephaly observed in all patients, indicating the otherwise normal gross structural development and preservation of the nervous system and validating the assignment of specific metabolic abnormalities by PET to underlying brain regions (Figs. 1 and 2).

**Discussion**

Regional Uptake Deficits Caused by GLUT1 Haploinsufficiency

Our data on the decrease in cerebral glucose uptake coupled with the findings of hypoglycorrhachia and the syndromic association of cognitive and motor impairment, acquired microcephaly, ataxia, and seizures in these patients confirm the postulate that the predominant defect in Glut1-DS is the reduced entry of glucose into the brain. Genetic analysis of the GLUT1 gene showed mutations in all the patients, establishing the primary molecular defect and the fundamental pathophysiological abnormality. Analysis of the pattern of cerebral glucose uptake did, however, show an unsuspected distribution. First, the deficits were markedly regional, and, second, the structures that showed lower uptake were not necessarily those conventionally thought to exhibit greater glucose metabolic rates. Additional imaging studies using MRI documented the integrity of the nervous system in Glut1 deficiency and discounted the contribution of gross structural abnormalities to the FDG-PET findings.

Regional cerebral pathology in genetic metabolic diseases is a rather common occurrence and usually is explained by a variety of hypothetical mechanisms that might account for the relative sparing of some areas and overt dysfunction or even necrosis of other areas. The selective expression of the gene in question, the importance of a particular metabolic pathway regulated by the gene product in a cellular population, the existence of complex genetic interactions, and the presence of compensatory mechanisms are processes that have been invoked in these region-sparing diseases. However, the fundamental purpose that the entry of glucose into all brain structures serves appears, at least at first sight, irreconcilable with these hypotheses.

Although the expression of GLUT1 is detectable in the blastocyst, the presence of Glut1 in the human brain before the 10th postconceptional week has not...
been documented. At that stage, however, all cerebral regions are enriched in Glut1. At 17 weeks, Glut2 appears in the cerebellum, diencephalon, and metaencephalon. Glut5 is detectable in the cerebellum on the 10th week. Glut3 is also present in the adult human brain and is considered the main neuronal glucose transporter. In addition to the regional diversity of the Glut isoforms, their cellular and subcellular location is also specific. For example, GLUT1 is much more densely expressed in human cerebral capillaries from the 12th to the 18th postconceptional week and predominantly redistributed to the abluminal endothelial surface during that period, whereas the neuropil does not appear to parallel this increase in expression. The abundance of transporters other than Glut1 therefore may provide an explanation of our finding of regionality. Several, although not entirely applicable here, experimental pathological findings in animals and humans who suffered acute and severe hypoglycemia confirm the vulnerability of all the cerebral structures affected in our patients (ie, cortex, thalamus). Nevertheless, the pattern of injury in acute hypoglycemia (centered on the neocortex, hippocampus, caudate nucleus, cerebellum, and associated hydrocephalus) does not parallel our observations in Glut1-DS, suggesting differential mechanisms of insult and/or adaptation. Exemplifying the discrepancy between acute (acquired) and sustained (genetic) neuroglycopenia, the basal ganglia exhibit cellular loss after the acute injury, whereas the PET signal in Glut1-DS shows a relative increase over the surrounding structures. Therefore, the state of genetic neuroglycopenia cannot be deemed a milder and more prolonged variant of acute hypoglycemia.

Primary and Secondary Metabolic Deficits
The FDG-PET signal is particularly sensitive to activation by increased afferent input, allowing the elaboration of functional human cerebral maps. The robust connectivity between the most affected regions in our study (thalamus and cortex) poses another potential...

Fig 1. Comparison of 18F-fluorodeoxyglucose (FDG) uptake in Glut1 deficiency and normal control and cranial magnetic resonance images of the same Glut1 patient. (A–C) Axial, parasagittal, and coronal FDG–positron emission tomography (FDG-PET) images of a normal 20-year-old male subject. The parasagittal view includes the left medial temporal lobe. Physiological distribution of the radiotracer is appreciable. (D–F) Analogous images in Glut1 deficiency. Patient L (see Table), one of the Glut1-DS index patients was subject to FDG-PET. The radiotracer distribution appears globally diminished in comparison with the normal subject, except for an apparently increased uptake by the basal ganglia. (G, H) Parasagittal and axial T1-weighted magnetic resonance images of Patient L selected approximately at the level of the FDG-PET images shown. Note the normal configuration and appearance of the brain. FDG-PET images were pseudocolored from red (maximum uptake) to dark blue and black (minimal uptake) for display purposes.
mechanism to explain the distribution of abnormalities based on functional deafferentation. Decreased metabolism in a circumscribed region may conceivably result in decreased signal output through its projections, resulting in a change in the activity of the innervated targets and therefore in their metabolic rate. This mechanism, known as diaschisis, was first postulated to explain the decreased state of arousal induced by lesions in the reticular activating system (the brainstem reticular formation) and recently has been subject to detailed investigation in focal human lesions by functional imaging methods. In particular, thalamic ischemic lesions have been associated with cortical hypometabolism by FDG-PET. We propose therefore that partial and permanent functional deafferentation may accentuate the defect in cortical metabolism that we found.

Uniformity of Deficits across Childhood
The results show that the pattern of decreased uptake exhibits little variation among our patients, regardless of degree of affection, history of seizures, diet, or age. The presence of all the metabolic abnormalities that constitute the imaging signature of the disease in our youngest patient suggests that, by age 19 months, the brain suffers the full burden of Glut1 deficiency and that development fails to provide significant compensatory mechanisms to remedy the abnormalities detected by our methods. Among the first structures to display metabolic activity by FDG-PET in the newborn are the thalamus, the sensory and motor cortices, the cerebellar vermis, and the brainstem. Our results are compatible with an early (perhaps prenatal) effect of the disease on the structures that first start to mature, that is, the thalamus. In support of this hypothesis, FDG-PET studies of children 2 to 6 years of age who suffered perinatal anoxia have shown a pattern of glucose uptake similar to that of the normal neonate, suggesting that cerebral maturational arrest is associated with perpetuation of the pattern present at the time of injury.

Epilepsy and Thalamocortical Metabolism
Diminished glucose uptake in certain cerebral regions has been implicated in epileptogenesis, because seizure foci often exhibit metabolic alterations. Of these, the best known involve cortical dysplasias and the cortex of the mesial temporal lobes. Our patients displayed cortical FDG-PET abnormalities, with more profound involvement of the mesial temporal lobes. There did not appear to be an obvious correlation between seizure history and the severity of the decrease in FDG-PET signal we found. In the 55 Glut1-DS patients we have studied, when epilepsy is still a feature of the disease (eg, before the initiation of a ketogenic diet), the characteristic electroencephalographic change is a generalized 2.5 to 4.0Hz spike-wave pattern, with little focality specific to the temporal lobes (data not shown).
Focal epileptiform discharges have been found in infants, but this finding evolves into the generalized spike-wave discharge in late infancy or late childhood. Experimental observations in humans afflicted with absence type epilepsy and subject to intracerebral electrical recording and in animals with comparable pharmacologically induced seizures have suggested that the interactions between the rhythmically excitable cells of the thalamus and the cerebral cortex serve as the generator of a generalized 2.5 to 3.5Hz spike-wave rhythm observed in the electroencephalogram. The hypometabolism of thalamus and cortex found in our patients speaks to the severe abnormality of this functional system and leads us to hypothesize that it constitutes the central epileptogenic substrate in Glut1-DS.

Pathogenesis and Physiological Consequences of Neuroglycopenia

Our findings offer the first direct evidence that the disease impairs the uptake of glucose by the brain. The mechanisms by which mutation of the GLUT1 gene disrupts the function of the Glut1 transporter are multiple and depend on the particular mutation. In one of the most severe forms of deficiency, one entire allele is missing, owing to deletion. In other cases, the allele harbors point mutations or deletions that result in a protein that terminates prematurely and does not retain functional activity or that remains trapped in the intracytoplasmic membranous network, unable to reach the plasma membrane. We have evidence for these phenomena in several of our patients (data not shown), who tend to be severely affected. Of particular interest are missense mutations that interfere with functional aspects of the operation of the transporter, such as the affinity for the substrate or the velocity of transport. These patients range across a wide spectrum of phenotypic severity, presumably because of the degree of residual activity of the transporter, although our results on this correlation are preliminary (unpublished).

The direct consequence of transport insufficiency is the increase in transcellular glucose gradients (between blood and endothelium, endothelium and astroglia, and perhaps astroglia and neurons) and the limited response capacity under situations of extra metabolic demand. Glucose in the astrocyte serves primarily two functions, apart from any direct transport into neurons: the maintenance of glycogen stores and the supply of energy associated with neural transmission. Astrocytic glycolysis may serve as a source of energy for neurons, perhaps via breakdown into glucose or lactate, during periods of excessive functional demand and/or short supply of glucose and as a partial substrate for nicotinamide adenine dinucleotide phosphate regeneration via the pentose phosphate pathway. In synergy with this hypothesis, activation of astrocytic β-adrenergic receptors stimulates glycogenolysis. Neural transmission involves the uptake of glutamate by the astrocytes at most central nervous system synapses. In vitro cellular and functional imaging (functional MRI and PET) evidence is compatible with the contention that the uptake and recycling of glutamate into glutamine is coupled to the uptake and aerobic breakdown of astrocytic glucose, to be later served to the neuron in the form of lactate. If this hypothesis is correct, neurotransmission cannot proceed expeditiously under conditions of limited glucose availability because the cycle is slowed down and neuronal ATP production from lactate is diminished. An additional process dependent on glucose is DNA synthesis and repair, which is contingent on the function of the pentose phosphate pathway via the production of ribose-5-phosphate. Elucidation of the participation of this mechanism to the pathophysiology of Glut1-DS will require further study.

Conclusion

Our results demonstrate that the primary defect caused by mutation of GLUT1 is the impairment of transport of glucose into the brain. Exploration of the pathophysiology of the disease will shed light on fundamental processes such as the utilization of glucose by the brain, the interaction between neurons and glia, and the regional maturation of the nervous system. The appearance of the abnormalities characteristic of the disease at an early stage of development emphasizes the value of prompt recognition and initiation of therapy and the utility of FDG-PET in the diagnosis of Glut1 deficiency.

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References


