A. Study Purpose and Rationale

Glut1 Deficiency Syndrome is an autosomal dominant defect of the type 1 glucose transporter present in the blood-brain barrier and is characterized by persistent hypoglycorrhachia, infantile-onset epilepsy, acquired microcephaly, movement disorder, and developmental delay 1,2,3,4. Diagnosis of Glut1 Deficiency Syndrome is confirmed by an abnormal erythrocyte glucose uptake assay or a pathogenic mutation in the SLC2A1 gene encoding Glut1 protein. In 2011, our team studied 109 patients with suspected Glut1 Deficiency Syndrome who presented with the classical phenotype and low cerebrospinal fluid glucose 5. Of these patients, 75 had decreased glucose uptake on the erythrocyte uptake assay and/or a pathogenic mutation in the SLC2A1 gene, essentially confirming the diagnosis of Glut1 Deficiency Syndrome. Of the remaining 34 patients, 15 were diagnosed as having transient hypoglycorrhachia of infancy, based on normal neurological development and resolution of neurological symptoms at the time of publication in 2011. In the other 19 patients, the diagnosis remains unclear, as they continued to have persistent neurological symptoms at the time of publication. In our clinical experience, these patients have tended to have severe chronic disease consistent with the phenotype of Glut1 Deficiency Syndrome. This leaves the question of whether these patients may have mutations in different genes that produce phenotypes similar to Glut1 Deficiency without directly changing the Glut1 protein, such as mutations in genes that regulate Glut1 protein in the blood-brain barrier. Conversely, it is unclear why the symptoms spontaneously resolved in the group with transient hypoglycorrhachia of infancy. Similar to infantile reversible cytochrome c oxidase deficiency, recovery may be due to developmentally time-sensitive processes, such as switching of a pervasive fetal subunit for a more ubiquitous subunit, or modifying effects of other developmentally-regulated genes 6. Furthermore, it has not yet been confirmed that all of these patients continued to have normal neurological development, nor that all 19 patients with persistent symptoms continued to have neurological symptoms later in childhood. An update on this total group of 34 patients would provide better information to permit clinicians to give clear and accurate prognosis and treatment suggestions to this group of patients with infantile symptoms suggestive of Glut1 Deficiency Syndrome but a negative work-up as described above. Furthermore, due to the likelihood that different molecular mechanisms are responsible for the presenting symptoms in the group with transient hypoglycorrhachia compared to the group with unclear chronic neurological conditions, there may be a biological marker present early on that could help predict the diagnosis and, therefore, the prognosis for individual patients. Cerebrospinal fluid glucose level at initial presentation may be such a marker as it may be significantly different in the patients with transient hypoglycorrhachia as compared to the patients with chronic conditions. Other possible prognostic factors, or possible confounders, may be age at initial presentation and cerebrospinal fluid lactate value. Not only would investigation of these concepts assist with prognostic information, but it could lead to suggestions of possible pathogenic mechanisms leading to each condition and could therefore assist with further work designed to more specifically elucidate such mechanisms.

Hypotheses:

1) No individual patients will meet criteria to cross-over from the transient hypoglycorrhachia group to the unclear chronic condition group, or vice versa.

2) Initial CSF glucose will be significantly different between the group with transient hypoglycorrhachia and the group with an unclear chronic condition.
B. Study Design and Statistical Analysis

This study will consist of two parts. One part will be a cross-sectional description of diseases, as a follow-up to the cross-sectional description presented in our team’s 2011 paper. This cross-sectional description will be obtained by contacting parents of the 34 subjects initially enrolled in the study in 2011. Information will be obtained from parents using a standardized telephone interview as well as a qualitative discussion. We will first ensure that the disease categories into which patients were placed in 2011 (either transient or chronic) continue to be accurate for each individual subject. To be included in the transient hypoglycorrhachia of infancy group, subjects must meet the following criteria: 1) No more than 1 delayed developmental milestone since age 2 years; 2) Complete resolution of any seizures and movement disorders by age 2 years; and 3) No new neurological symptoms since age 2 years. Any subjects who were categorized as having transient hypoglycorrhachia of infancy in 2011 but do not meet the above criteria on follow-up, or who were categorized as having unclear chronic conditions in 2011 but do meet the above criteria on follow-up, will be considered to have “crossed over” between groups.

The second part of this study will involve statistical evaluation of whether cerebrospinal fluid glucose on initial presentation is significantly different between groups. There are two groups in this study: subjects with transient hypoglycorrhachia of infancy and subjects with unclear chronic neurological conditions. Groups will be determined based on whether subjects meet criteria for transient hypoglycorrhachia of infancy on follow-up interview. Since we hypothesize that no subjects will have crossed over from one group to another since 2011, we assume that there will be 15 subjects in the transient hypoglycorrhachia group and 19 subjects in the unclear chronic group. An unpaired two-tailed t-test will be used for statistical analysis. Alpha will be set at 0.05. With 80% power, we will be able to detect a difference of approximately one standard deviation in the means of the cerebrospinal glucose between the two groups as statistically significant. Since the standard deviation of our initial cerebrospinal fluid glucose levels is about 5.6 mg/dL, there will have to be at least a 5.6 mg/dL difference in the averages of the two groups for us to detect it as significant. In addition to an unpaired t-test on this variable, we could do a covariate analysis that takes into account age at symptom onset as well as cerebrospinal fluid lactate levels on presentation. We could first ensure that any difference we detect with an initial t-test on CSF glucose levels remains present when these covariates are accounted for. We may also be able to develop, through regression analysis, a model that has prognostic significance based on a combination of these covariates.

C. Study Procedures: Study subjects were initially referred by their physicians due to concern for Glut1 Deficiency Syndrome. Subjects were then confirmed to have a clinical and laboratory phenotype consistent with Glut1 DS prior to undergoing testing. Once phenotype was confirmed, subjects had an erythrocyte glucose uptake assay and mutation analysis of the Glut1 gene. This has been previously described 5.

For the follow-up study that we are now undertaking, we will contact via telephone the parents of the 34 subjects from the prior study who had normal erythrocyte glucose uptake assays and normal mutation analysis of the Glut1 gene. Informed consent for the 2011 study, as well as permission to contact these parents for further research, has already been obtained. Informed consent from parents and assent from minors, where applicable, will be obtained again to participate in this follow-up. The follow-up will consist of a standardized telephone
interview with parents, as well as an open-ended qualitative telephone discussion of their child’s development.

D. **Study Drugs: Not applicable**

E. **Medical Devices: Not applicable.**

F. **Study Questionnaires:** A standardized telephone interview will be used when contacting parents. It will include questions about the patient’s current age and presenting symptoms. It will also include a list of about 10 developmental milestones; parents will be asked about timing of these milestones to determine whether patients met those milestones on time, delayed, or not at all. It will also include a list of movement disorders commonly associated with Glut1 Deficiency Syndrome to see if patients had these disorders, and, if they did, whether or not they resolved. It will include questions about seizure occurrence, frequency, and whether and when seizures resolved, if at all. It will also include a list of maladaptive behaviors commonly associated with Glut1 Deficiency Syndrome to see if patients had these behaviors and, if so, whether or not they resolved. Finally, there will be an open-ended qualitative discussion at the end of the standardized interview so that parents have the opportunity to tell us anything else of interest or concern to them about their child’s development.

G. **Study Subjects:** Subjects for this study will be those subjects who participated in our team’s earlier study in 2011, who were found to have normal erythrocyte glucose uptake assays and no identified mutation in the SLC2A1 gene. Initial inclusion criteria for the 2011 study were patients who were referred for evaluation for Glut1 Deficiency Syndrome in whom the following criteria for suspected Glut1 Deficiency Syndrome were met: 1) Clinical findings: infantile-onset seizures, spastic ataxia, delayed neurological development, dysarthria, acquired microcephaly, paroxysmal movement disorders; and 2) Laboratory findings: normal blood glucose values (~70-110mg/dl), low CSF glucose values (<40mg/dl), normal or low CSF lactate values (<2.2mM). All clinical findings were not necessarily present in each case, but all had early delays in neurological development. Minors will be included because the symptoms of the conditions being investigated present in the infantile period, and the subjects who were included in the initial study in 2011 are still minors. It is also important that we include minors in this study because one of our goals is to improve prognostic information regarding prediction of neurological development in childhood. Informed consent from parents and assent from minors was obtained with the initial study. Parents and minors will be required to provide updated informed consent and assent, respectively, to participate in this follow-up study.

H. **Recruitment of Subjects:**

Subjects will be recruited from our database with previous study subjects. They will be approached about the follow-up study by phone call. Initial recruitment for the 2011 study was done by recruiting patients who were referred by their primary physicians.

**Draft of Phone Call Information:** We are calling from Columbia University Medical Center because your child participated in a research study of patients with suspected Glut1 Deficiency Syndrome a few years ago. We are wondering if you and your child are willing to participate in a follow-up to that study. The purpose of this follow-up study is to better understand the conditions which can present like Glut1 Deficiency Syndrome but which are, in fact, are different entities. This information would enable us to give better information in the
future to families of children with suspected Glut1 Deficiency Syndrome but a negative work-up for that disease. The follow-up study requires a telephone interview with you (the parent) during which we will ask you about your child’s development and any neurological symptoms, such as seizures or movement problems, that he or she has experienced.

I. **Confidentiality of Study Data:** All study data will be coded and de-identified. Study subjects will be assigned a unique code number. Paper data will be stored in a secure, locked location on the Child Neurology floor. Electronic data will be stored only on locked devices. All data will be accessible only to the investigators.

J. **Potential Conflicts of Interest:** None.

K. **Location of the Study:** Child Neurology division at New York Presbytarian Hospital, Columbia University Medical Center.

L. **Potential Risks:** There is no more than minimal risk to study subjects in this study. The only risks are of minimal inconvenience to parents to take the time for the phone interview, and the risk of disclosure of protected health information. The risk of disclosure will be minimized as specified in the “Confidentiality of Study Data” above. Should any breach of confidentiality occur, it will be immediately reported to the IRB with a plan for corrective action.

M. **Potential Benefits:** The results of this study could lead to better diagnostic and prognostic guidelines for patients who have symptoms similar to Glut1 Deficiency Syndrome but a negative work-up. These results will be of value to parents and clinicians taking care of future children who meet the above criteria. The results may also lead to better understanding of the mechanisms behind conditions which are similar to Glut1 Deficiency Syndrome, which may lead to better treatment recommendations in the future.

N. **Alternative Therapies:** Not applicable.

O. **Compensation to Subjects:** None.

P. **Costs to Subjects:** None.

Q. **Minors as Research Subjects:** This study involves minors. Approval for the initial study has been obtained from the Department of Pediatrics Committee on Human Investigation.

R. **Radiation or Radioactive Substances:** Not applicable.

**References:**


