



# Poster Session

## July 11, 2019



### **#1 Follow Up of 8 Cases With Glucose Transporter Type 1 (Glut1) Deficiency Syndrome: Diagnosis and Nutritional Treatment with Ketogenic Diet**

Veronica Cornejo

**INTRODUCTION:** Glucose Transporter type 1 (GLUT1) deficiency syndrome is an inborn error of glucose transport. Clinical manifestations are secondary to reduced glucose transport across the blood brain barrier, including: refractory seizures, developmental delay, microcephaly, persistent hypoglycorrhachia with normal glycemia. The diagnosis is suspected with glucose CSF/glycemia  $<40$  (NV: $> 60$ ) and confirmed by molecular study in SLC2A1 gene (cr1p35-31.3). Treatment is based on a ketogenic diet (KD).

**OBJECTIVE:** To present the Chilean experience in diagnosis and follow-up of patients with GLUT1.

**METHODOLOGY:** 8 records of patients with GLUT1. Clinical picture, biochemical exams, molecular study, nutritional status and macro and micronutrient with KD were recorded. **RESULTS:** 5/8 are men. Of the total 4/8 GLUT1 were diagnosed with median (Me) 9,0 years of age and 4/8 GLUT1 with (Me) 5,3 months. All had hypoglycorrhachia  $<40$  mg/dl (range:13-30) without hypoglycemia (80-90 mg/dl). The CSF/blood glucose ratio was (Me) 0,33. At the time of diagnosis, they presented myoclonic seizures, ataxia, and paroxysmal movements without response to drugs. They started DK and it has stayed between 18 years to 1 month. The seizures ceased after md 5 days of starting KD in children who were diagnosed at (Me) 5,3 months. The distribution of the caloric molecule was: Lipids: 87-85% (MCT oil, alpha linolenic acid and docosahexaenoic acid), Protein: 10-8% (0,8 to 2,0 g/wt/day), Carbohydrates: 3-6 %. Fasting levels of beta-hydroxybutyrate acid has been maintained over 2,0 mM/l and after meals lower 5 mM/l. The fasting blood sugar level was (Me) 80 mg/dL (NV: 60-90), Total cholesterol: (Me)141 mg/dL (NV: $<200$ ), LDL: (Me) 77 mg/dL (NV: $<100$ ), HDL: (Me) 62mg/dL (NV: $>40$ ), Triglycerides: (Me) 63.6 mg/dL (NV: $<150$ ), Vitamin D serum: (Me) 45,3 ng/ml (NV: $>38$ ), potassium: (Me) 4,4 mEq/L (NV:3,4-4,7). All of them are supplemented with L-carnitine, vitamin C, sugar-free multivitamins, calcium. 7/8 GLUT1 have molecular study (C.177del.p; c.1088G>A; c.420delG; c57delA; 969del-C971T; c.143G>A; c.458G>C/p.R153P). According to nutritional status: 3/8 are eutrophic, 1/8 have malnutrition, 3/8 are overweight and 1/8 are at risk of malnutrition.

**CONCLUSIONS:** In patients with refractory epilepsy, GLUT1 deficiency screening and early diagnosis is important considering that KD is a safe and effective treatment for improving neurological manifestations.

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### **#2 Effect of Alzheimer's disease associated mutations and amyloid-beta, glucose deprivation and ketone bodies on the barrier function and glucose uptake at the blood-brain barrier using a stem cell derived in vitro model of the blood-brain barrier.**

Abraham Al-Ahmad

**Introduction:** The blood-brain barrier (BBB) plays an important role in the brain homeostasis, by providing a physical and chemical barrier against xenobiotics and noxious compounds. Yet, the blood-brain barrier plays an

important role by being the major entry point of glucose (the major source of energy in the brain). As of today, glucose homeostasis and metabolism at the blood-brain barrier remains unclear, however several studies demonstrated evidence of its alteration in Alzheimer's disease and Glut1 Deficiency syndrome. In addition, the effect of ketone bodies on the BBB function remains unclear. In this study, we investigated the impact of Alzheimer's diseases associated features (PSEN1, PSEN2 mutations; amyloid-beta), glucose deprivation and ketone bodies on the barrier function and glucose uptake. Methods: iPSC lines from controls (CTR90F, CTR65M, CTR06F, CTR88M) and from early onset of Alzheimer's (FAD08F, FAD40M) were differentiated into brain microvascular endothelial cells (BMECs) following established protocols. BMECs barrier function was assessed by trans endothelial electrical resistance (TEER), fluorescein permeability, immunocytochemistry, transporter activity was assessed by accumulation assay. Glucose deprivation stress was obtained by challenging cells with glucose-free media for 24 hours. Ketone bodies supplementation was performed by adding 3mM acetoacetate (AC) and/or 5mM beta-hydroxybutyrate (BHB) to glucose-free medium for 24 hours. Results: Our data demonstrate that glucose deprivation significantly alter the barrier function in iPSC-derived BMECs, such disruption was not recovered by the presence of ketone bodies. In addition, we noted a significant decrease in cell metabolic activity in those cells, suggesting an important contribution of glucose to BMECs energy supply. Notably, preliminary data showed an alteration of glucose uptake by AC treatment but not by BHB treatment. In addition, presence of a mutation in PSEN1 gene (FAD40M) yielded to an impaired barrier function and glucose uptake compared to controls or PSEN2 mutant (FAD06F). Similar effects were observed by treating control cells with amyloid beta peptide 1-40 for 48 hours at concentrations ranging from 1nM to 300nM.

**Discussion:** Our preliminary data suggest that BMECs heavily rely on glucose to maintain their barrier function, such deficit appears not corrected by supplementation with ketone bodies. Furthermore, PSEN1 mutation yielded a deficit in the barrier function compared to other cell lines, as we have noted an impaired barrier function and glucose uptake. However, similar observations were not reported in PSEN2 mutant iPSC line. We speculate that PSEN1, not PSEN2, may have an important function on the blood-brain barrier and may yields to its dysfunction. Finally, preliminary data observed in our BMECs showed a decreased GLUT1 protein expression and glucose diffusion following Abeta 1-40 treatment in a dose-dependent manner. Taken together, our data suggest that glucose maybe an important source of energy for both the blood-brain barrier and the central nervous system. Furthermore, Alzheimer's disease features observed in early and late onset suggest a possible dysfunction via glucose metabolism impairment.

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### **#3 Food Cost of Classic Ketogenic Diet in Patients with GLUT1 Children's Mercy Hospital, Kansas City**

**Rachel Finn**

**Introduction:** Glucose Transporter Deficiency Syndrome (GLUT1) is a rare genetic metabolic disorder characterized by deficiency of a protein that is required for glucose to cross the blood-brain barrier. The current gold standard treatment for GLUT1 is the ketogenic diet (KD). The KD is carefully tailored to individual patients and involves purchasing specific foods unique to the family member on the KD. The foods and supplements required on the KD are currently an out of pocket expense for the majority of families (minimal insurance coverage). A review of literature indicated the average cost of the specialty foods and supplements to families is unknown. The purpose of this study was to determine the estimated monthly cost of KD ingredients of foods and supplement consumed by orally fed patients with GLUT1.

**Methods:** This study involved a retrospective chart review of patients seen in the Epilepsy Center at Children's Mercy Hospital (CMH) with the diagnoses of GLUT1 between 4-24 years of age who were orally fed on the classic KD. These patients are on the medically prescribed classic ketogenic diet which provides the recommended energy for their diagnosis. Patient's current meals, snacks and supplements were analyzed for the

cost of the ingredients the families purchase. Cost of the ingredients was determined through visits to local grocery stores, averaged and then used for the calculations. The use of a ketogenic diet calculation program was used to determine the total grams of ingredients the patients consumed through pre-calculated meals, snacks and supplement for each patient. Calculation parameters include age, gender, diet ratio and calories were compared to the USDA food plans: Cost of Food at Home at the liberal plan for a family of four.

**Results:** Nine patients with GLUT1 were identified on the KD at CMH. Average monthly cost of foods purchased was \$143-185 per patient on the KD compared to USDA estimated average monthly cost food plan for a family of four \$42-82. Average cost was not associated with age, gender, calories nor ratio. Cost of ingredients reflected on the types of foods the patient consumed. Micronutrient supplement costs ranged between \$0.22-2.22 per day per patient.

**Conclusion:** The average cost of ingredients for GLUT1 patients on the KD is 1.75-3.5 times greater than the USDA average for age/gender for a liberal food plan. The KD foods and supplements are currently an out of pocket expense for most families. Future research will include multiple sites representing additional regions of the United States. The data collected can provide information for potential future legislation or program development intended to support families' expenses related to the dietary treatment of their disease.

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#### **#4 Exploring the Genotype(s) Associated with Glut1 DS-Like Phenotypes: A Case Study**

##### **Sarah Park**

Classical Glut1 Deficiency Syndrome (Glut1 DS) is characterized by infantile onset seizures, eye movement abnormalities, delayed neurological development, acquired microcephaly, disturbed cerebrospinal fluid (CSF) biomarkers including low glucose and lactate concentrations, and diminished uptake of glucose in vitro by the patient's freshly prepared red blood cells (RBC) (De Vivo 1991; Klepper 1999). This RBC uptake assay generally predicts the presence of a disease-causing SLC2A1 mutation (Seidner 1998; Yang 2011). Yang et al (2011) studied 109 patients who had a clinical phenotype consistent with Glut1 DS and confirmatory CSF biomarkers. Seventy-four (68%) patients had a low RBC uptake assay and 72 (97%) of these patients had a documented SLC2A1 pathogenic mutation. In contrast, only 1 (3%) of the 35 patients with a normal RBC uptake assay had a documented disease-causing mutation in the SLC2A1 gene. These uptake studies allowed us to predict that the clinical phenotype and associated CSF biomarkers were caused by a pathogenic mutation in the SLC2A1 gene. If normal, it strongly suggested a different disease mechanism as has been described recently with the PURA gene (Mayorga 2018). We have introduced the term "Glut1 DS-Like" to describe this group of patients. We have studied about 30-40 patients with the Glut1 DS-Like phenotype. All have normal RBC uptake assays and SLC2A1 gene studies as determined by Whole Exome Sequencing and MLPA analysis. We have studied one representative patient in detail over several years as emblematic of this overlapping phenotype. The patient was first seen at the Columbia University Irving Medical Center at age 9 months with generalized seizures, delayed neurological growth and development, and microcephaly. The first major convulsion occurred at age 3 months. A ketogenic diet (KD) was started at age 5 months with cessation of seizures 8 days later. The KD was discontinued one year later because of kidney stones. CSF glucose concentrations were low (21, 21, 30, 31 mg/dl) on 4 lumbar punctures with correspondingly low-normal lactate concentrations (0.8, 0.8, 1.0 and 0.9 mM). However, the RBC glucose uptake assay was normal (time curve: 113%, Vmax: 129%, Km: 99%), and genetic testing failed to identify a pathogenic mutation in the SLC2A1 gene. Microarray analysis and sequencing of the SLC2A3 (Glut3) gene also were negative. PET scan analysis showed a global decrease of cortical glucose metabolism, most marked in the thalamus, and relatively preserved in the basal ganglia. MRI scans showed diffuse cortical atrophy and EEG analysis showed diffuse and multifocal cerebral dysfunction. Cultured skin fibroblast analysis showed decreased expression of Glut1 protein (62%) and Glut1 RNA transcript (~67%), and above normal RBC glucose

uptake (111-153%). The patient died at age 7.5 years and autopsy was refused. This representative case study implies another disease mechanism(s) contributing to a Glut1 DS-Like phenotype with hypoglycorrachia. The recent report of hypoglycorrachia, a Glut1 DS-Like phenotype and a pathogenic mutation of the PURA gene is further support for remote molecular influences on SLC2A1 gene expression. Hopefully, the continued investigation of novel gene-gene interactions will lead to new opportunities regarding additional therapeutic targets.

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## **#5 Current status of ketogenic diet therapy in patients with Glucose Transporter 1 Deficiency Syndrome in Argentina**

**Marisa Armeno, MD**

Glut1 deficiency is a metabolic disease, and the KD (ketogenic diet) is considered the first-line therapy for this syndrome, since ketones use another transporter to enter the CNS providing the brain with an alternative source of fuel, correcting the impaired brain energy metabolism. We conducted a survey on the ketogenic diet therapy in 14 patients diagnosed with glucose transporter protein-1 deficiency syndrome (GLUT1 DS) to evaluate the efficacy of the treatment with the classic ketogenic or modified Atkins diet from the viewpoint of the patients' families. A 3-page questionnaire was distributed among all attendees of a family-centered meeting for GLUT1 DS held in November 2017 at Hospital Pediatria Garrahan, Buenos Aires, Argentina. Patients were from Santa Fe, Neuquen, Rio Negro and Buenos Aires, Argentina. The questionnaires were completed by parents and collected anonymously. Information was collected in a database and analyzed. Questionnaires were received from 14 families of patients diagnosed with GLUT1 DS. Four patients were female and 10 male with a median age 7.8 years (3 to 18). Three patients were diagnosed before one year of age, and the maximum age at diagnosis was 11 years. Eleven patients started treatment within 6 months after diagnosis. Most patients were hospitalized and fasted for treatment. The mean diet duration was 8 years (range, 5 months - 16.5 years). The types of KD therapy used were the classic KD (n: 10) and the modified Atkins diet (n:4). The most commonly used ratio was 4:1, and in most patients (13 of 14) urine ketones were measured twice a week. All parents were concerned about the level of ketones and noted a relationship between ketosis and movements, behavior, and learning. None of the patients but one reported adverse effects related to the KD (diarrhea). Two patients who had reached puberty showed changes in behavior, stability, and a decreased level of ketones, despite treatment. All patients were supplemented with multivitamins and 64% with carnitine. Only one was receiving MCT at that moment. The families complained about the late diagnosis but they showed a high level of satisfaction with the efficacy of the KD therapy. Although the long-term prognosis in patients with GLUT1 DS partly depends on the underlying genetics, our study supports the assumption that early initiation of treatment with a ketogenic diet is feasible and may positively affect outcome.

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## **#6 Starting the low-glycemic index ketogenic diet during pregnancy in an asymptomatic mother of a GLUT1 patient**

**Marisa Armeno, MD**

**Introduction:** Glucose Transporter Type1 Deficiency Syndrome (Glut1DS) is a rare genetic disorder resulting in impaired brain metabolism leading to delayed growth and function. The KD (ketogenic diet) is the first-line therapy for this syndrome, providing ketones as an alternative fuel to the brain, correcting the impaired brain

energy metabolism.

**Case report:** The mother of a child with Glut1DS, diagnosed at age 2 years and responding well to the KD, becomes pregnant again. A molecular test is requested showing her to be an asymptomatic Glut1DS carrier. Amniocentesis to detect Glut1DS in the fetus revealed the same heterozygous mutation in the SLC2A1 gene (c1408G>C) as in the mother and the sister. The mother is put on a prophylactic low-glycemic index diet (LDID, with carbohydrate restriction up to 60 gr/day) at week 25 continuing until the end of the pregnancy. Ketosis was achieved on the diet. During the pregnancy, the mother has suffered from nausea, anemia, low B12, and low carnitine levels for which she received supplements. Echo Doppler ultrasonography at 37 weeks was normal. On July 15 a cesarean section is planned.

**Conclusion:** Ketosis may be achieved with a low ketogenic index during pregnancy as a prophylactic treatment for Glut1DS for the fetus. Carnitine deficit should be monitored.

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## #7 My Life With Glut1

### JR Rapaport

I will describe my life with Glut1. This will include photos as I was growing up, a timeline of important milestones that were good and bad and a graph of my sanity over time.

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## #8 A Sibling Odyssey

### Gabbie Meisner, Erin Meisner, Walter Meisner

Taking a personal tone of reflection, this poster highlights some of the more recent research and first-hand experience to outline several various aspects of the life of a sibling to a patient with Glut1 Deficiency Syndrome. The authors attempt to identify some of the challenges facing siblings, while also focusing on the benefits and potential positive outcomes of the experience. Understanding and providing context to the sibling's parallel journey is a powerful reminder that a diagnosed patient does not proceed in isolation.

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## #9 Celebrating Successes of Glut1 Deficiency Patients

Joanna Snyder and her family created a special project to highlight the achievements and special talents of our children and adults with Glut1 Deficiency. It is a source of celebration and inspiration to see them reach milestones and do things that many of us were told our loved ones with Glut1 might not ever be able to do.

Thank you!

to all who presented and visited