



# 2011 Glut1 Deficiency Conference

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## Conference Summary Report

prepared by Kris Engelstad  
Columbia University Medical Center  
Colleen Giblin Research Laboratories for Pediatric Neurology

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## “Mutation Spectrum and Therapy for GLUT1 Deficiency Syndrome”

There are 3 billion letters in our genetic code and 1 change can cause a health problem.

DNA is made of individual nucleotides (like letters in a book).

DNA consists of exons (*the set of nucleotides that codes for the protein*) and introns (*the set of nucleotides that don't code for the protein*).

There are 2 types of laboratory testing to evaluate whether a patient has Glut-1 DS:

- 1) Red blood cell glucose uptake - this is a very good test using blood from the patient and from controls (*people who don't have Glut-1 DS*). Few laboratories run this assay.
- 2) Genetic testing- DNA evaluation including : DNA sequencing, MLPA, Chromosome microarray/exon array.

Diagnostic yield of GLUT1 Deficiency Syndrome

If you have normal RBC glucose uptake you likely do not have Glut1 DS.

If you have abnormal RBC glucose uptake you likely have Glut1 DS.

Typical mutations

Deletions, missense, nonsense, splice site.

Deletions- some or a lot of the DNA is missing.

Missense- the genetic “letters” are switched from one letter to another

Nonsense- the mutation creates an incorrect message in the DNA that tells the cell to stop making GLUT1 protein from that gene (*the GLUT1 gene that doesn't have the mutation **will** make Glut1 protein*).

Splice Site- the mutation causes an incorrect message in the DNA so that normal “pruning” of the DNA doesn't happen.

### Glut1 DS Symptoms

There is some correlation between the type of mutation and clinical severity, however this is not perfect.

There is a wide spectrum of Glut-1 DS symptoms. Some individuals have a GLUT1 mutation and have no or almost no symptoms. However, there are other patients who have a mutation and are clinically severe.

Clinical types of symptoms that are seen in Glut-1 DS can include:

- 1) Movement disorders with no seizures.
- 2) Epilepsy (seizures) with no movement disorders.
- 3) Movement disorders and epilepsy.

Within individual families there can be symptom variation between members who are carriers of the same mutation, so environment may play somewhat of a factor.

### Mode of inheritance of the GLUT1 gene.

#### Autosomal dominant

GLUT1 gene mutations are usually de novo. This means that the mutation occurred first in the patient and no other person in the family had the mutation prior to the patient. Each time you have a child about 20 genetic mistakes are made in the DNA that the parent doesn't have. The patient with a GLUT1 mutation can pass the mutation to his/her child. There is a 50-50 chance of passing down the gene mutation to each child. Each child is an independent event.

#### Germline mosaicism

A GLUT1 gene mutation occurred in sperm or eggs of the parent and it can be passed to the child. The risk of inheritance varies depending on how many of the sperm/eggs carry the mutation. This is rare and could account for about 1% of cases.

#### Autosomal Recessive

Both parents carry a copy of the genetic mistake and each parent passes this to the child. Each child has a 1 in 4 chance of having Glut1 DS. Generally Glut-1 DS is not recessive, however there are a few cases of this. The inheritance pattern is determined by haploinsufficiency. The two parents have normal cognitive function and somewhat lower RBC glucose uptake, but are still in the normal range. The child who inherits these two mutations has much lowered RBC uptake and is symptomatic (has Glut1 DS).

#### Another situation

A child inherits a mutation from a parent and develops another mutation on the other GLUT1 gene. This is also rare but has been seen.

### Newborn screening for Glut-1 DS

Newborn screening (NBS) for Glut1 DS would be ideal for this disorder. NBS is normally done for all babies by getting a blood sample through a heel stick. It would be nice to screen for Glut-1 DS so that the child would start the ketogenic diet earlier. It is estimated that the frequency of Glut-1 DS is 1/100,000 individuals. Currently this is an issue because most families have a new genetic mutation, so NBS can not be done by just looking for a couple of common mutations as is the case in some other diseases. It helps to advocate for NBS as for Glut-1 DS in order to potentially have it added to the NBS panel.

### New Genetic Test on the Horizon

A new panel of genes for genetic testing in seizure patients should be available by end of 2011. The GLUT1 gene is on this panel. This is useful, in that the test could be performed on anyone who has seizures. Thus, more patients would be diagnosed with Glut-1 DS.

## Family Planning Options

In this age of genetic testing there are many options available to people who carry a GLUT1 gene mutation.

### Amniocentesis

Performed at 16-20 weeks

Very reliable 1/1000

Pregnancy loss rate is 1/200 to 1/400

Available and easy to perform (a sample of amniotic fluid is obtained)

Results come late

### CVS

Biopsy from chorionic villus

Performed at 10-12 weeks of pregnancy

Results come much sooner

Higher procedure related loss (miscarriage)

### Preimplantation genetic diagnosis - "test tube baby"

Woman takes hormone injections

Obtain eggs from the mother/sperm from the father

Fertilize in the laboratory

Genetically test one cell from each of the fertilized eggs

You can choose to transfer the egg that does not have the GLUT1 gene mutation

## Therapeutic implications for Glut-1 DS

### Diet

Ketogenic diet

Avoid caffeine, phenobarbital, barbiturates, chloral hydrate, tricyclic antidepressants

### Other potential strategies

Increase expression/mobilization of Glut 1 protein or stimulate insulin cascade

Alpha lipoic acid/ limited effect

Acetazolamide

Chaperone therapy-other clinical trials

Block degradation of Glut-1 protein (keep the Glut-1 protein from being normally destroyed)

Read through a GLUT1 gene mutation-prevents a mutation from prematurely stopping the process of making proteins.

Gene therapy put in a new Glut1.

Some of these options are being studied in other disorders, so they could possibly be used in Glut-1 DS if they are successful.

If a mutation hasn't been found in your family, try to get genetic testing again as there may be new technology to identify a gene mutation.

## “Glut-1 Deficiency Syndrome”

A world without the disorder of Glut-1 DS is achievable.

### What is normal growth and development?

In infancy, the ratio of brain size to body size is much larger than in an older child or an adult. The proportion is 1:10 at birth. By 3 years of age, the brain size is almost the size it will be for life. Glucose utilization by the brain increases with as the number of nerves in the brain increase. There is a rapid increase from birth to 3 years of age. For the 1st decade of life, glucose utilization is elevated and then drops off during the 2nd decade of life. Furthermore, it drops off again in adulthood.

### Neurological domains affected by Glut-1 DS

- 1) Cognition-learning and memory.  
Intellectual disability ranges from mild to severe in Glut-1 DS patients.
- 2) Behavior-attention and social relatedness.  
Glut-1 DS patients are very friendly, some have ADHD (Attention Deficit Hyperactivity Disorder), many have seizures.
- 3) Movement-motor activity and posture.  
Spasticity, ataxia, dystonia.

### Classic Glut1 DS Phenotype

The classical Glut-1 DS phenotype is a developmental encephalopathy. (*The word phenotype means the characteristic set of symptoms associated with a disease.*) The phenotype includes: Infantile presentation with seizures, developmental delay, and microcephaly (small head size) and hypoglycorrhacia (low CSF glucose).

Currently we are seeing more mild phenotypes and we expect to see a lot more in the future.

### Key Point #1

Glucose transport is important for brain development thus Glut-1 DS is poorly tolerated. The brain prefers to use glucose as an energy source. The brain will also use ketones (from fats) as a source of energy. Since glucose transport into the brain is insufficient in Glut-1 DS, fats should be used as a source of energy. Early diagnosis is important because the child can be placed on the ketogenic diet to maximize growth and development.

The Glut1 DS phenotype is changing and the diagnostic criteria for Glut-1 DS is changing. Historically we recognized the classical phenotype (noted above). More recently we have encountered other phenotypes which include epilepsy and movement disorders.

The phenotypic spectrum of Glut-1 DS is expanding.

- 1) Not all patients with Glut-1 DS have seizures.
- 2) It is questioned whether all patients have hypoglychorrachia? The answer is yes, but this is debatable in a few patients
- 3) Low CSF glucose and clinical symptoms are not enough to make a diagnosis of Glut-1 DS.
- 4) The RBC glucose uptake assay is important in diagnosing patients, however we know of a few patients with Glut-1 DS who have normal uptake
- 5) Not all patients with Glut-1 DS have mutations in the GLUT1 gene.

Various phenotypes already seen in Glut-1 DS include:

- Alternating hemiplegia of childhood
- Absence epilepsy
- Paroxysmal exertion induced dyskinesia (PED)
- Episodic ataxia
- DYT9 may be Glut-1 DS

Lumbar punctures should be performed if there is a neurological condition that can't otherwise be explained.

### Key Point #2

Establishing the diagnosis with certainty.

- 1) Epilepsy is an ascertainment bias.  
If clinicians only test for Glut-1 DS in patients with seizures, then the patients who have Glut-1 DS but no seizures will be missed.
- 2) Hypoglychorrachia is sensitive but not specific.  
We know from our studies that most Glut-1 DS patients have low CSF glucose (sensitive marker of disease) but there are also patients with low CSF glucose who don't have Glut-1 DS (not always specific to Glut-1 DS).
- 3) The RBC assay is a more sensitive test than genetic analysis (*looking for a GLUT1 gene mutation*).
- 4) Normal CSF glucose is >60 and generally about 65 mg/dl.  
91% of Glut-1 DS patients are below 40 mg/dl.  
9% of Glut-1 DS patients are between 40-50 mg/dl.

We don't really know what the normal CSF glucose is because patients have lumbar punctures only when they are sick. We can assume that <60mg/dl is likely abnormal.

### Key Point #3

We need a “Standard of Care” document.

A uniform “standard of care” document should be created by the experts in Glut-1 DS, as there is great variability in which patients are managed. This document will describe the best way to clinically manage a patient. This is especially useful for disorders such as Glut-1 DS because many health care professionals will have only a few Glut-1 DS patients, so they aren't familiar with treating the disorder.

Management principles:

Nourishing the developing brain is important. The ketogenic diet is the standard of care for this disorder. The ketogenic diet is important to not only stop seizures, but also to nourish the brain with enough ketones to function at an optimal level. Checking the urine ketones is not an acceptable measure of ketones. The blood ketones must be checked.

### Key Point #4

Developing a patient registry is important.

The creation of a patient registry is important as a useful resource for clinical trials. It would provide easy access to patients for enrollment into research studies and clinical trials.

### Key Point #5

Researchers need to continue to look for newer therapeutic measures.

### Funding

Funding is always an issue with all of the researchers. Obtaining grant funding through the National Institute of Health is becoming more and more difficult.

## “Cognitive and Behavior Skills in Children with Glut1 Deficiency Syndrome”

The GLUT1 gene is responsible for making the glucose protein. The job of the Glut1 protein is to transport glucose into the brain. This ultimately affects the structure and the function of the brain, resulting in cognition and behavior.

Seizures affect function of brain and the outcome can result in a change in cognition and behavior.

### Intellectual function in Glut-1 DS patients:

Normal population average IQ is 100. Glut-1 DS kids have a wide range of functioning, but tend to fall below the average IQ.

### Verbal skills in Glut-1 DS patients:

Communication skills- receptive language (*understanding what someone else has said*) is stronger than expressive language (*expressing self through speech*).

Poor articulation/dysfluent speech (*speech is uneven*)

### Non verbal skills:

We can test these skills which don't require the patient to speak

Tests include: drawing, matching, peg placing and fine motor skills.

Most kids fall below the average range, but some children are above the normal functioning.

### Cognitive processing styles in general:

- 1) Sequential or local processing-absorbing information bit by bit (see the trees before the forest).
- 2) Simultaneous or global processing-synthesize information (see the forest before the trees).

Glut-1 patients do better with sequential processing task

In higher functioning Glut-1 DS kids there is a primary reliance on sequential processing



### Adaptive behavior (how does a person function in the real world?)

A person might do poorly on an IQ test but might be able to function well in the real world.

Adaptive behavior and IQ are positively correlated- if you have more IQ you will have more adaptive behavior.

Social skills are considered part of adaptive behaviors. Social skills are relative strengths in the Glut-1 patients. Glut1 DS parents report that their children are warm and affectionate, playful, empathetic, humorous, considerate, interested in others, and well liked.

### Behavioral Issues

Children with developmental delay tend to have a higher incidence of behavior problems overall. However, if we look at the data about the Glut-1 DS children, we do not see any increased frequency in unusual behaviors in the kids compared to children without Glut-1 DS. Furthermore, behaviors are not associated with neurological score (i.e. if a Glut-1 DS child has a worse neurological score than another Glut-1 DS child, he/she is no more likely to have behavioral problems). It should not be assumed that behavioral issues that are seen in the Glut-1 DS patients are caused by the Glut-1 DS. In general, behavior in Glut-1 DS patients is within normal limits.

Glut-1 DS patients may be at risk for adjustment difficulties. This could be even more so with higher functioning more insightful children. What we have seen seems to be normal. It doesn't seem to be related to Glut-1 DS.

In Glut-1 DS, IQ is associated with neurological function and it is positively correlated. This means that if your neurological functioning score is higher you are more likely to have a higher IQ also (lower IQ scores are associated with lower neurological function).

Cognitive performance is not associated with age or gender in Glut-1 DS.

We can make no strong statements regarding IQ and mutation type.

### Performance across time

Developmental gains are made with time.

Relative functioning compared to peers seems to be stable over time.

There is no evidence of progressive decline over time.

Cognitive skills tend to be the same over time.

Adaptive behavior tends to stay the same over time.

Behavior skills stay the same over time.

What are the implications of these findings?

- 1) Skills vary between Glut1 DS children.
- 2) Your child is unique.
- 3) Because of communication difficulties children can be judged as less intelligently than they are. Thus, targeting speech skills is important.
- 4) Visual search skills should be used in training these kids. For example try tests such as “spot the difference in this picture”.
- 5) Go over your child’s homework.
- 6) Since fine motor skills are weak, physical therapy and occupational therapy can help. The use of technology to help communicate could be of benefit.
- 7) Use teaching strategies that rely on sequential of step-wise approach (give bit by bit).
- 8) Rely on rote memorization and repetition.
- 9) Break large assignments into manageable steps. Consider using organization strategies similar to those recommended for kids with ADHD .
- 10) Give kids a little extra time/help when trying to pull together the whole picture.
- 11) Play with abstract puzzles where they have to pull together the sum of the parts to make a whole picture.
- 12) Work on adaptive behavior skills- give them chores at home and hold them to those standards.
- 13) Social skills are strengths- encourage kids to be active in school groups and all social groups.
- 14) There is no aberrant behavior associated with Glut1 DS. There may be inattention and adjustment concerns related to living with a developmental disability.

## “Clinical Genetic testing for GLUT-1 DS (SLC2A1)”

A clinically validated SLC2A1 DNA test exists and is used to diagnose Glut-1 DS.

The NIH sponsors the NIH CETT program which was designed to help “mainstream” rare research based DNA tests for use in clinical laboratories. This means that if a researcher develops a DNA test for a known disease, the NIH will help in the process of getting clinical laboratories to offer the test on a commercial basis.

Research and develop of new technology requires the ability to clinically validate the assay prior to implementing the test for the diagnosis of patients.

An example of the research and discovery process:

- 1) The research laboratory discovers and/or characterizes SLC2A1 mutations in the research setting.
- 2) The researcher must analyze whether the test is useful.
- 3) The clinical laboratory must obtain CLIA approval prior to offering the test as a diagnostic measure and for the “sale” of this product.
- 4) The clinical laboratory must also validate that when the test is performed it is performed accurately from one sample to the next.
- 5) This all takes time and money.

When DNA testing is performed it generally covers only certain parts of the potential DNA that could affect the protein production and function.

The patient charge at UT Southwestern for genetic testing is \$2,700. Different insurance companies will pay differing amounts for genetic testing. The wait time for genetic testing results varies.

Positive DNA sequencing tests are informative in that a mutation is found.

Negative DNA sequencing tests means that a mutation wasn't found. If the patient has clinical symptoms but DNA testing doesn't show a mutation, this does not rule out the possibility of a genetic mutation elsewhere in the gene.

Types of DNA gene mutations that are generally not analyzed by most laboratories:

- 1) Far promoter mutations (areas of DNA that are not within the GLUT1 gene but could affect whether the gene is used to make Glut-1 protein)
- 2) Deep intron mutations (areas of DNA within the GLUT1 gene that do not code for protein but could affect whether the gene is used to make Glut-1 protein).
- 3) Mutations on either end of gene (DNA outside of the GLUT1 gene that may affect the GLUT1 gene expression)

There are national regulatory trends for genetic testing. The current regulating bodies include: CLIA and FDA which regulate both gene and genomic testing.

CLIA- Clinical laboratory results must be performed in a CLIA approved laboratory.

FDA- whatever you report must be clinically significant. Traditionally the FDA hasn't been involved with DNA testing, but newer regulations are being developed. This will raise cost of genetic testing, but hopefully will standardize and hopefully improve the quality of testing.

### The genetic information non discrimination act (GINA)

GINA is a law that was established by the federal government to protect people who have had genetic testing.

What is covered under GINA:

Prohibits genetic predisposition based denials or higher premiums for health insurance.

Prohibits employers from using genetic predisposition information as a reason to not hire someone.

Health insurers and employers cannot request for existing predisposition genetic information or that genetic testing be performed.

*(note: genetic predisposition means that you carry a gene mutation that has been known to increase the risk of having a disorder, but currently do not have symptoms of disease).*

What is not covered under GINA:

If you have already have a genetic disease.

Companies with <15 employees.

Life insurance can request for this information/testing and deny coverage.

Disability insurance can request for this information/testing and deny coverage.

Children's Medical Center – Dallas has a CLIA/CAP approved genetic testing for sequencing the GLUT1 gene (SLC2A1).

Genetic information is kept in the hospital's electronic medical record which can be accessed by the patient's physicians as well as insurance providers.

## “Glucose Transporter Type I Deficiency Syndrome: 2010-2011 in Review and 2011-2012 Agenda”

The laboratory has expanded its clinical and basic research personnel and members are involved with the following: MR spectroscopy, human biochemistry, genome diagnostics (DNA analysis), neuropsychology.

Basic research projects include: mouse electrophysiology, brain tissue samples.

### What has been accomplished?

- 1) Funding for C7 study was established.
- 2) Established the ability to run EEG's and video monitoring on mice.
- 3) NIH funding for normal brain metabolism studies of which some have been published.
- 4) A smaller grant for brain metabolism was funded (this involves general brain metabolism which includes Glut1-DS).
- 5) A grant application for brain metabolism in Glut-1 DS (mice) was submitted and received high scores.
- 6) A grant proposal was submitted to the “Network for Excellence in Neuroscience Clinical Trials”.
- 7) A grant proposal was submitted in collaboration with the North American Mitochondrial Disease Consortium. This is a proposal for research into brain metabolism.

### There are three parts to disease research:

- 1) Testing-the doctor must be able to run a test to see if the patient has the disorder. The Office of Rare Disease has set up standard clinical (commercial) DNA testing for patients and families at UT Southwestern.
- 2) Understanding-the doctor must understand the nature of the disorder.
- 3) Treating-once the doctor knows that the patient has the disease, and understands the basic nature of the disorder, treatment options can be utilized or developed.

### Update on C7 (triheptanoin) study:

The ground work has been established in the past year. Human studies have not yet been done. We hope to initiate studies by the end of 2011.

We took advantage of the information obtained in glycogen storage disease study. In this disease, glucose turns to glycogen (*normal process*), however, glycogen is not broken down further (*not normal*).

We are planning on doing this type of research in mice but first we need to know the mechanism. We need to know what the effects are on brain metabolism. We will be looking at MR (magnetic resonance) technology. Normally, the brain is given glucose and we look at many of the chemicals that are then formed in the brain. But we want to know what happens in Glut-1 DS mice and in patients.

We want to know what happens with triheptanoin (C7). The mice are given triheptanoin through an IV in the neck. We know that the krebs cycle (TCA cycle) is fundamental in the process of breaking down glucose for its eventual production of ATP(energy). The 6 carbons in glucose are taken up by brain and are metabolized (made into other chemicals). Neurotransmitters are made from glucose (and some other chemicals).

In Glut-1 DS, glucose metabolism is normal. There are abnormalities in the synapses (where neuro-transmission happens). Communication between neurons happens in the synapse. But what happens when you give triheptanoin? In order to answer this we can give it to mice, analyze the metabolism, and look at neurotransmitters. Glucose is a precursor (building block source) to a lot of transmissions. C7 seems to be making more transmitters in the Glut1 mouse, as expected and desired.

What happens in cell to cell communication in the context of C7? C7 also seems to help. We can evaluate synapses in the context of glucose and tripeptanoin. We can look at electrical currents between the neurons. Neuronal control includes both excitation (*making a neuron "fire" an electrical impulse*) and inhibition (*inhibiting that exectrical impulse from "firing"*). Inhibition is suppressed in Glut-1 DS in the mice. Inhibition increases in the context of ketone bodies and triheptanoin has shown evidence to do this as well.

A patient registry is essential for Glut-1 DS.

A patient registry is an asset to clinical trials.

Registries make it easier for clinical researchers to find patients for all studies.

Registries allow researchers to learn more about patients because additional information is available.

#### Patient Registry Issues

- Compatibility with NIH guidelines.

- Any research project is heavily regulated by HIPPA and the IRB.

- There are issues of confidentiality, safety of data, etc.

- The registry will be voluntary (patients can decide if they want to participate).

- Worldwide possibility to participate.

- Life-long commitment for patients (progress can be updates).

- Information and care resources can be linked to the registry.

Studies for the upcoming year:

- 1) Start clinical trial with C7 for Glut1 DS before end of 2011.

We are waiting for IRB approval.

Patients are already lined up for the studies.

Inclusion criteria

Patient must have a DNA mutation in the GLUT1 gene.

Patients must not be on the ketogenic diet (C7 will interfere with the diet).

Able to travel to Dallas 2-3 times.

Study related procedures

Medical exam

MRS-brain metabolism studies (oxygen consumption)

Neuropsychological studies (cognitive ability)

Blood samples

- 2) A study of brain metabolism in Glut-1 DS is open for recruitment.

Inclusion criteria

Glut-1 DS diagnosis proven by DNA mutation in the GLUT1 gene.

Patients can be on any diet

Able to travel to Dallas

Exclusion criteria

Child can't lie still for 15 minutes without sedation.

Patient has metal in body (cochlear implant, VNS, etc)

Study procedures

Medical exam

MR spectroscopy for brain metabolism (oxygen consumption)

Neuropsychological studies (cognition)

Cost

Most costs are covered but call center to verify.

## “Epilepsy in Glut-1 DS”

### Outline

- 1) Excitation and inhibition
- 2) Energy failure
- 3) Treatments

### 1) Basic Definitions

A seizure is a sudden surge of electrical activity in the brain that usually affects how a person feels or acts within a short time. Many different disorders can cause seizures.

Epilepsy is the tendency towards having seizure, or when a person has 2 or more seizures on 2 occasions, separated by at least 24 hours. Additionally, these cannot have been caused by a direct injury or intake of harmful substance. Epilepsy is not a disease itself, but is a symptom caused by a number of different diseases.

Seizures can look different in one individual and between individuals. For example, they could be emotional responses, behavioral actions, and some seizures are very subtle in appearance and may not be detected except using video EEG.

Seizures may have many different manifestations:

- Chewing movements
- Breathing difficulty
- Drooling
- Falling down
- Fluttering of eyelids
- Sensory manifestations such as
- Confusions, black outs, smells, spacing out, fear, panic

Please see [www.epilepsy.com](http://www.epilepsy.com) to see examples of these types of seizures.

### 2) Excitation and Inhibition & Energy Deficit

Any person can have a seizure if the right circumstances prevail. What protects our brains from having seizures?

#### Excitation and Inhibition

There are two types of neurons in the brain - inhibitory and excitatory

Excitatory neurons - amplify the signals

Inhibitory neurons inhibit signals

We need both for a normally functioning brain.



### Energy Deficit

To keep these neurons in balance we need a source of energy - without the energy to keep this in balance seizures can result. In Glut-1 DS we do not have enough energy supply (glucose) to the brain to balance out the excitatory activity, thus seizures result.

What do seizures in patients with Glut-1 DS look like?

90% of individuals with Glut-1 DS have seizures starting in infancy. These are often focal (involving limited areas of the body/brain) and can also be generalized (involving both sides of the body).

Seizures are variable in Glut-1 DS. In some individuals, they can occur very frequently, or only once in a while. Seizures may occur in individuals who also have developmental difficulties and movement disorders.

### Possible seizure manifestations:

- Whole body stiffening
- Brief staring events
- Brief jerk of a limb
- Seizures associated with falling - possibility of injury

### **3) Treatments**

How do the seizure types respond to seizure medications?

We treat the Glut 1 DS seizures by treating the condition of energy failure. Traditional seizure medications are not effective and do not combat the problem of energy failure.

Some medications can make this energy failure worse, such as caffeine and benzodiazepines.

The standard treatment for Glut-1 DS is the ketogenic diet, which treats all symptoms of the condition. The ketogenic diet is high in fat and low in carbohydrates which forces body to burn fat which as an alternative fuel source.

Generally, the outcomes of using ketogenic diet in Glut-1 DS are:

- Rapid improvement of symptoms
- Increased alertness and activity
- Withdrawal of other anti-epileptic drugs

The ketogenic diet should be started as early as possible and maintained through puberty to allow for proper development.

## “Why do we need to perform the RBC Glucose Uptake Assay?”

Glut1 Deficiency Syndrome (Glut-1 DS) was first described by Dr. De Vivo et al., in 1991. Since then, more than 400 patients have been diagnosed world-wide. Glut1 DS is characterized by early onset seizures, developmental delay, acquired microcephaly, cognitive impairment, spasticity, ataxia, and dystonia. The phenotype (characteristic set of symptoms) has expanded significantly in the past few years. Paroxysmal presentations have been described, including paroxysmal exercise induced dyskinesia – DYT18, alternating hemiplegia of childhood, early onset absence epilepsy, and dystonic tremor. The biochemical hallmark of Glut1 DS is low CSF glucose.

Glucose is the primary fuel source for brain metabolism. It is water soluble and transferred across the blood-brain barrier into the brain by a mechanism called facilitated transport. This facilitated transport is mediated by glucose transporter protein type 1 (Glut1), which belongs to a Glut family consisting of 14 members (Glut 1-14).

Glut1 is expressed predominantly in the blood-tissue barrier such as blood-brain barrier (BBB), and the red blood cells. The BBB consists of endothelial cells of capillaries connected by tight junctions (*which means that they are tightly held together so that chemicals can not just flow from one cell to another*). Glut1 is expressed both on the luminal and abluminal membrane of the endothelial cell. In order for glucose to get into the brain, it needs to cross the luminal membrane into the endothelial cell, then cross the abluminal membrane into the intracellular space of the brain. Astroglial cells also express Glut1. Both the endothelial cell and the red blood cell express the same type of Glut1, which is the molecular basis of our red blood cell uptake assay which was developed to confirm the clinical diagnosis of Glut1 DS.

The GLUT1 gene (also known as SLC2A1) is located on chromosome 1. It is about 35kb (35,000 nucleotides of DNA) long, consisting of 10 exons (*protein coding portion of the gene*), and 9 introns (does not code for protein). There are 492 amino acids in the Glut-1 protein. *Amino acids are the building blocks of a protein. Just like a train is composed of individual cars, so too a protein is made of individual amino acids (“cars”) all hooked together.*

An overall evaluation of the RBC assay was performed at Columbia University. In this study, 109 suspected Glut1 DS patients (58 females, 51 males; age range from 2 months-26 years; mean age 6 years) were selected based on the following criteria:

- 1) Clinical findings: early-onset seizures, spastic ataxia, delayed neurological development, dysarthria, acquired microcephaly and paroxysmal movement disorders.
- 2) Laboratory findings: normal blood glucose levels (~70-150mg/dl), low CSF glucose levels (<52 mg/dl), and a normal-to-low CSF lactate level (<2.2mM).

As a result of this study, the cut-off for an abnormally low uptake value was increased from the previous value of 60% to 74%. This study was important in that allowed us to diagnose additional patients with Glut1 DS. Based on this new cut-off value, 74 patients were found to have decreased 3-OMG uptake (group 1) and 35 patients had normal 3-OMG uptake (group 2). The mean uptake for group 1 was  $54.2 \pm 8.9\%$  (Mean $\pm$ SD, n = 74) with a median of 55% (range 37-72%). The mean uptake for group 2 was  $107.9 \pm 19.1\%$  (Mean  $\pm$  SD, n = 35) with a median of 104% (range 74 -175%).

We identified a pathogenic GLUT1 mutation in 70 out of 74 group 1 patients and one out of 35 group 2 patients with a heterozygous missense mutation (T295M) in the GLUT1 gene. This mutation specifically alters Glut1 conformation (*shape*) and minimally affects glucose influx, but significantly abolishes the glucose efflux across the cell membrane. Structure-function studies, such as this, explain the seemingly paradoxical observation of Glut1 DS with hypoglycorrhachia (*low CSF glucose*) and “normal” erythrocyte glucose uptake. The erythrocyte uptake assay (RBC assay) only measures the influx activity.

In this study, we also showed that the average RBC uptake values were inversely correlated with the severity of the phenotype (*as CSF glucose levels are decreased, disease severity increases*). The RBC assay has 99% sensitivity and 100% specificity. *Sensitivity is a measure of the degree to which a test is positive (has an abnormal lab value) in the patient population. Specificity is a measure of the degree to which a control (someone without the disease) does not test positive. A good test has a high degree of sensitivity and also specificity.* The RBC assay is a functional test of the Glut1 transporter activity. It can be used as the “gold standard” to confirm the clinical suspected Glut1 DS.

Notes by Kris Engelstad

# Question and Answer Session

## Grant applications

Grant applications are sent to NIH. Some of them are thrown out right away. If they are not thrown out they get sent to study section. There is study section and peer review. They vote on the final outcome. The senate doesn't get involved with this but they are influential in setting the budget.

## Why isn't there a lot of money for gene therapy when it can help so many different kinds of diseases?

About 10 years ago there was a lot of work on this issue but a young man died when treated with gene therapy in the clinical trial. There were a lot of regulations implemented to try to make gene therapy research safer. Now there are new vectors for putting genes into the body. They have been useful in the laboratory in mice, but that doesn't mean that they will work or be safe for humans. The reason why this doesn't happen faster is that there have been other successes and failures at times, some kids have developed leukemia because of this. Researchers are trying to be responsible and still move this along.

## Should my child be on thioctic acid?

This is another name for alpha lipoic acid which we have been recommending.

## How can we help get this information to our doctors?

Spreading the message can be one of the missions of the families and Glut-1 organizational group. The doctors speak at meetings and write articles for medical journals, however this is a slow process. This is approaching a critical mass in that more physicians know about Glut-1 DS.

## Standard of care documents.

Dr. Chung- "Standard of Care" documents are very important. This includes recognizing the condition, diagnosing it and managing it. It should be created for Glut-1 DS.

## Is the ketogenic diet enough to really replace glucose? Do we know anything about being able to reverse the structural change in the brain?

The MRI's are generally normal in kids with Glut-1 DS. However, the whole size of the brain is generally lower than in children without Glut-1 DS. If a child has a smaller brain than average, the brain size could increase as a result of the ketogenic diet. The use of glucose is different in different parts of the brain which may account why we see different types of seizures at different ages.

## If we find a fix for Glut-1 DS can the damage be reversed?

The best tool that we have to answer this problem can be by evaluating the mouse brain. In principle this could be reversible, but the studies need to be done in the mouse.

Regarding brain structure and function, the brain's structure is normal even in patients who are severely affected. The function is not normal due to chemical imbalance (i.e. less glucose). In general the principle of "Equipose" is important: if you have two approaches to something that you are going to do you should not favor treatment A or B. There is no evidence that Glut-1 DS isn't reversible. We have some evidence in other disorders that the phenotype can be rescued.