2012 Glut1 Deficiency Foundation Conference
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Conference Summary Report

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Slides from breakout sessions available at www.G1DFoundation.org or at the links below:

Dr. Mary Ciccarelli – Building Towards Success in Adult Life

Laura Kinney, MS, RD and Jenny Kramer MS, RD - Ketogenic diet: How to Make it Work for Life

Rylin Rogers – Family Resources Tool Kit: Understanding the Funding Maze

Dr. Cigdem Akman
Professor of Neurology
Columbia University
New York City, NY

Glucose Transporter 1 Deficiency Syndrome and Epilepsy

Classical phenotype as shown in two Glut-1 DS patients.

Patient 1
First seizure at 2.5 months of age
Seizure control with anti-epileptic medications failed
Head circumference was reduced
Low CSF glucose
Seizures stopped with use of ketogenic diet

Patient 2
Frist seizure 2 months
Initial EEG's were normal until 27 months of age
Low CSF glucose
Increasing spasticity and ataxia after 1 years of age
Seizures stopped with use of ketogenic diet

Cardinal features of Glut-1 DS

1) **Developmental delay**
2) **Seizures**
3) **Movement disorders**

Seizures common to Glut-1 DS
Brief, subtle limb jerk, staring spells, eye rolling, sudden pallor, unresponsiveness, horizontal roving eye movements, limpness, head nodding

Non-seizure events in Glut-1 DS

- **Intermittent ataxia**
- **Periodic confusion**
- **Periodic weakness**
- **Limb paralysis**

The medical records of 87 Glut-1 DS patients were reviewed to assess seizure activity
78/87 patients had seizures
Age at seizure onset was an average of 8 months
Seizure types were varied

The order in frequency for seizure occurrence in Glut-1 DS was: (Most frequent) Absence---Complex partial---Myoclonic---Drop---Tonic---Simple partial---Epileptic spasms---Infantile spasms (Least frequent)

Anti-epileptic drug (AED) treatment- many AED's drugs are tried with little or no success. Many patients have tried 3 AED's with little or no success. The ketogenic diet is currently the standard of care to treat seizure activity.

Seizure onset generally starts before 1 year of age. However the average age for a lumbar puncture is 4 years. Thus, initial diagnostic testing happens much later than symptom onset. The ketogenic diet is generally started soon after a lumbar puncture indicates low CSF glucose. Genetic testing to confirm a diagnosis generally happens much later.

In a set of patients at Columbia University, the ketogenic diet was used in 64 of 78 patients.
60% responded and were seizure free within 1 week.
Within a 1 month period 75% were seizure free.
Of Glut-1DS patients treated with AED's (rather than the ketogenic diet) less than 10% become seizure free.

<u>Seizure presentations vary</u>
Epilepsy diagnosis also varies (focal or generalized).
There is no correlation between genotype and seizure types.
The EEG may be normal in some Glut-1 DS patient. Furthermore, the EEG findings are not diagnostic meaning that they are not unique to Glut-1 DS.

Clinical spectrum of epilepsy with Glut-1 DS
Mild epilepsy to severe epilepsy.
Mild generalized epilepsy---focal---refractory generalized---myoclonic astatic epilepsy—intractable infantile seizures

Three types of epilepsy described in mild epilepsy phenotype
CAE-child absence epilepsy
JAE-juvenile absence epilepsy
JME-juvenile myoclonic epilepsy

It is possible to have different seizure symptoms within a family and the clinical course (i.e what happens over time) may be different.
Milder phenotype or atypical phenotypes may have seizures later than the classical phenotypes.

<u>EEG findings in Glut-1 DS</u>
The EEG is not diagnostic
Baseline EEG activity is slower
Focal or generalized spikes are noted
EEG's may improve with carbohydrate enriched food intake
EEG may be normal in 17% of cases

EEG's obtained before a glucose (sugar like substance) intake are more erratic then 1 hour after glucose intake. Furthermore, the EEG at 2 and 3 hours post glucose still looks "quiet". At 4 hours we start seeing more spike discharges (worsening EEG). Duration of spikes is longer pre glucose and after 5 hours. [Note: increased spikes or height of curves on the EEG indicate increased brain activity which can be seizures].

<u>Dietary Information</u>

A typical diet of western countries includes fat, protein carbohydrate. Most of the calories are from carbohydrates (eg. bread, pastas, fruits).
In the ketogenic diet the bulk of calories comes from fat.
Classical ketogenic diet includes long chain triglycerides as a source of fat. Brain can not use fats as a source of energy so the fatty acids (made from fats) are first metabolized in the liver where ketone bodies are produced and released to blood stream where they can go to the brain. Ketone bodies can meet 70% of brain's needs.

The ketogenic diet is important for treatment of seizures yet also for other cardinal signs of Glut-1 DS.

<u>Summary</u>
Seizures are the cardinal feature of the "Classical" and "Mild" Phenotype for Glut-1 DS
Seizure activity responds well to the ketogenic diet
Freedom from seizures exists in about 80% of patients treated with the ketogenic diet.
The ketogenic diet should remain the standard of care until something else is discovered that works at least as well or better.

Dr. Darryl De Vivo
Sidney Carter Professor of Neurology
Columbia University
New York City

Glut-1 Deficiency Syndrome:
A Treatable condition with several different clinical presentations

The original phenotype is called the "Classical Phenotype" (1991 article)

Signs and symptoms include:
1)Early onset of symptoms in infancy
2)Seizures or other paroxysmal events (may not be seizures)
3) Decreased brain growth post-natal
4) Hypoglychorrhachia (low CSF glucose)
5) CSF lactate low or normal
6) Decreased RBC glucose uptake assay
7) Therapeutic response to ketogenic diet

The ratio between CSF and blood glucose is less important than the absolute CSF glucose value.

Three major neurological domains are affected:

1) **Cognitive (intellectual disability)**
2) **Behavioral (ADHD, very friendly, epilepsy)**
3) **Movement (spasticity, ataxia, dystonia)**

The "Classic" phenotype is a developmental encephalopathy with all of the above domains affected proportionately.

There is no clear evidence of autism in Glut-1 DS patients. Social relatedness, in fact, is a relative strength

Summary of Glut-1 DS from 1991-2012

Clinical phenotypes have expanded to include several allelic variants

1) **First variant is dominated by epilepsy**
2) **Second variant is dominated by Movement disorders**
3) **Third variant is dominated Neurobehavioral disturbance (cognition and behavior affected disproportionally)**
4) **Fourth variant includes hemolytic anemia as a non-neurological manifestation of the Glut1 mutation**

The patient population at Columbia University
147 patients with Glut-1 DS
50% males /50% females
CSF glucose average is 33mg/dl (this is one-half of normal)
CSF lactate is 0.9mM/L (1.63 mM/L is normal)
CSF protein is 25 mg/dl
Red blood cell assay VMax is 50% of controls (parents)

Glut-1 DS Epilepsy Phenotype
10% of Glut-1 DS patients have not had a seizure or other heralding event
90% have seizures
68% of these present within the first 6 months of life
94% of these present within the first 2 years of life

The most common seizures (in order of decreasing frequency)
Generalized Tonic Clonic
Absence
Complex partial
Myoclonic
Drop attack
Tonic
Simple partial
Epileptic spasms
Infantile spasms

The earliest presenting patient was at 1 day. However, most patients have seizures within the first few months of life.
The average age of diagnosis is at about 5-6 years of age.
The average age of initiation of the ketogenic diet is about 4 years.
Many types of anti epileptic medications (AED's) were tried in patients most were ineffective.

Glut-1 DS Movement Disorder Phenotype (percent of patients with symptoms)
Ataxia -90% ("drunken walking")
Dystonia 85% (posturing or twisting)
Chorea 75% (dancing-like movements)
Tremor (70%)
Myoclonus (16%) (jerks)

Some previously known dystonic conditions are now known to be caused by Glut-1 DS
Paroxysmal exertional dystonia (DYT 18)
Familial choreoathetosis and spasticity (DYT 9)
Dystonic tremor
Alternating hemiplegia of childhood

Triggers of these movement disorders in Glut-1 DS: fatigue, excitement, dietary non compliance.

Glut-1 DS Cognitive Disability Phenotype
Estimated IQ for the group of Glut-1 children undergoing cognitive testing (n-63) is shifted downward by two SDs. However, some of the mildly/minimally affected patients are within the normal range for IQ.

IQ is correlates with neurological function (using the Columbia Neurological Score-CNS)
A worse neurological state is correlated with decreased IQ scores.

Glut-1 DS Hemolytic Anemia Phenotype
This is caused by a cation leak (ions leaking through cell membranes).
In these families, Glut-1 DS causes a Paroxysmal Exertional Dyskinesia (PED) and rupture of red blood cells.
In people without Glut-1 DS, sodium ions are largely outside of cell and postassium ions are largely inside the cell.
In hemolytic anemia, sodium ions leak inside and potassium ions leak outside of the cell.

How is the Diagnosis Confirmed?
What are the symptoms? Are they similar to known Glut-1 DS symptoms?
What is the relationship of the symptoms to meals?
PET scans show reduced glucose uptake in different brain regions.
Patients have a response to the ketogenic diet.
Lumbar puncture is performed and shows hypoglychorrachia.
Glucose uptake is reduced in the RBC assay.
Genetic testing for mutation in the SLC2A1 gene.

Columbia Neurological Score (CNS)
The CNS is a semi-quantitative tool used to summarize the clinical evaluation.
Scores range from 0-76 with no Glut-1DS patient scoring less than 40.
Severe phenotype scores 40-49
Moderate phenotype scores 50-59
Mild phenotype scores 60-69
Minimal phenotype scores 70-76

The general medical exam is mostly normal
Eye exam is mostly normal
Cranial nerve functions are mostly normal
Sensory examination is mostly normal
Pyramidal (motor) system is abnormal (spasiticity, stiffness difficulty with fine/gross motor skills)
Cerebellar (coordination) system is abnormal (difficulty walking a straight line)
Extrapyramidal (postural) system is abnormal (ataxia, dystonia, chorea, tremor)
With severity of phenotype- see worsening of pyramidal, cerebellar, and extrapyramidal tract signs

What is a normal CSF glucose?
We don't really know precisely because no healthy person gets a lumbar puncture.
90% of patients are between 20-40 mM/L
Most patients have CSF glucose under 50 mg/dl.
All patients have CSF glucose under 60 mg/dl.
On average the values are always low regardless of relationship to meals or time of day.

Red Blood Cell (RBC) Glucose Uptake Assay
Glut-1 protein is in the membrane of the RBC.
The assay is an influx assay, which means that it measures the rate at which glucose enters the cell.
Patients generally have 50% of the rate of the parents.
At the Colleen Giblin Laboratory RBC assays are performed if patient has clinical symptoms suggestive of of Glut-1 DS and a low CSF glucose.

In 109 suspected cases of Glut-1 DS at Columbia University
68% had low CSF glucose uptake
95% had a defined gene mutation

Whereas:
32 % had normal RBC uptake
<3% with a gene mutation in the SLC2A1 gene
These patients with hypoglychorrachia due to unknown reasons
Many had transient low CSF glucose as infants (benign hypoglycorrhachia of infancy).

The RBC uptake assay is both qualitative and semi quantitative.
In most cases, Glut-1 DS is considered an autosomal dominant trait. [Each person has 2 SLC2A1 genes and if 1 carries a mutation, the person will have Glut-1 DS]. RBC Uptake is generally around 50% of normal.

It is possible for families to have autosomal recessive mutations [both SLC2A1 genes must carry a mutation for the child to have Glut-1 DS].
In this case, parents with only 1 mutation have somewhat reduced uptake and have no symptoms.
The children who have 2 mutations have reduced uptake and symptoms of Glut-1 DS.

The main point is that somewhat reduced RBC glucose uptake is not clinically symptomatic if it is less than 25%.
Reductions of greater than 25% correlate clinically with Glut-1 symptoms.

Failure in brain energy metabolism causes a functional synaptopathy and disturbed connectivity.
Empirically, we prefer to treat early to help prevent structural changes and preserve brain function

"Nourish the starving brain" is the guiding principle.
The ketogenic diet is standard of care and blood ketone measurements should be around 5mM/L.

The future of Glut-1 DS
Breast feeding is likely neuroprotective.
Start the ketogenic diet as soon as possible after the clinical onset of symptoms.
New born screening would be useful, but isn't available at this point.
Therapeutically, the symptoms would lessen if the degree of haploinsufficiency were mitigated. RBC glucose uptake assay values greater than 75% are associated with clinically normal states. Values less than 74% are associated with increasingly symptomatic clinical states.
The classical phenotype is still the most common but now we are describing milder phenotypes.
Continue genetic testing.

Recent Publications

Acute hyperglycemia produces transient improvement in glucose transporter type 1 deficiency. Akman CI, Engelstad K, Hinton VJ, Ullner P, Koenigsberger D, Leary L, Wang D, De Vivo DC. Ann Neurol. 2010 Jan;67(1):31-40.

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Glut1 deficiency: inheritance pattern determined by haploinsufficiency.
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International Experiences with Glut-1 Deficiency Syndrome

slides from this presentation available at www.G1DFoundation.org or at the link here:

The focus of the talk is the European experience in Glut-1 DS.

Glut-1 Deficiency-What do we know?
Discovered by Dr. De Vivo in 1991.
Part of his team from 1997-1999
In 2012 there are over 200 patients known worldwide.
A German support group meeting/conference is held every year.
Glut-1DS is a worldwide disease with patients identified in many areas of globe.

The diagnosis is made by:

1) **Lumbar puncture**
2) **Fasting EEG—EEG worsens with brief fasting. Add food and see if it improves. (This is not seen in the minority of patients)**
3) **Glucose uptake assay**
4) **SLC2A1 gene—mutations are mostly heterozygous, most are de novo (only the patient has it), with autosomal dominant (rarely autosomal-recessive) inheritance.**

Genetic mutations:
Are important to know because of their functional significance.
Example: The Carboxy-tail of the Glut1 protein is important for substrate recognition. Mutations in the tail of the protein is like having the "wrong key" – glucose is not recognized anymore

Mutations can change the 3D structure of protein and result in "misfolding" of Glut1.
Microdeletion syndromes may involve several genes, sometimes including the SLC2A1 gene causing Glut-1 DS as one feature of a complex syndrome..

Symptoms of Glut-1 DS:
The Classic Phenotype includes encephalopathy, a complex movement disorder, and intractable epilepsy.
Other symptoms may include small head growth,

The Movement disorder includes:

- **Intention dystonia**
- **Chorea**
- **Intention tremor**
- **Dyspraxia**
- **Myoclonus**

Epilepsy includes:

- **Seizures are the most common presenting symptom.**
- **Cyanotic spells (infants)**
- **Absence (early onset below age 4 years)**
- **focal and generalized myoclonic (small jerks)**

The ketogenic diet is useful but doesn't cure everyone.

Subgroups of classic phenotype

1) **Movement disorders in the form of paroxysmal exertion-induced dystonia (PED) caused by brain basal ganglia dysfunction.**
2) **Epilepsy- could be in the form of absence seizures or myoclonic astatic ????**
3) **Paroxysmal non-epileptic events - 3 out of 4 patients surveyed reported such events.**
   Duration was from a few seconds to more than 10 minutes.
   There is no correlation between PED events and years on the ketogenic diet.
   With increasing age there is increased event frequency. In puberty there tend to be more events for reasons unclear.

4) Hereditary Hemolytic anemias- (Stomatin-associated cryohydrocytosis)
RBC leak potassium- cells burst and release potassium similar to PED.
Stomatin and Glut1 seem to interact in the protein membrane- mutation in SLC2A1 gene may affect stomatin

Epilepsy
1) 10% of children with early onset absence epilepsy <4 yrs carry Glut-1 mutations.
2) 5% of children with myoclonic-astatic epilepsy (also called "Doose Syndrome") carry Glut-mutations.

Treatment for Glut-1 DS

Glucose enters the brain and converts to Acetyl-CoA which enters the TCA cycle.
In the ketogenic diet free fatty acids convert to ketones via beta oxidation. Ketones are the alternative source for brain energy in times of fasting. Thus the Ketogenic diet offers Ketones as an alternative fuel for the brain, derived from nutrionial fat rather than body fat.

Ketogenic diets are:

- Ketogenic diet 4:1 fats:protein/carbohydrates (not in infants) recommended
- Ketogenic diet 3:1 fats/protein/carbohydrates recommended
- Modified Atkins Diet positive results
- Low glycemic index Diet no data

The taste improves as ketosis goes down in this list.

Possible adverse effects of ketogenic diet

Renal stones: patients who drink enough can generally avoid this. For those who don't drink enough potassium citrate is added to reduce the possibility of kidney stones. However, it has to be taken three times a day. We should monitor renal status by ultrasound every 6 months, if abnormal start potassium citrate.

Growth: growth rate is sometimes impaired on the ketogenic diet. Make sure the patient has sufficient supplements, increase calories, reduce ketosis by using a different diet (see above), potentially growth hormone is an option (personal experience in three cases).

Elevated lipids: this is a concern but long term data of patients using the ketogenic diet doesn't seem to indicate that it is a big problem. The lipid composition may be altered in the diet if it becomes a problem. We have seen cholesterol around the 200's and triglycerides are reduced slightly in patients monitored in Germany over 96 months.

Brain energy needs are highest through early childhood and levels out to adult demand in puberty, so patients should try to remain on the diet through puberty.

What we don't understand about Glut-1 DS

Glucose transport is not a one step process.
Glucose enters the Blood brain-barrier through the endothelial cell. There seems to be more Glut1 protein in the abluminal membrane then luminal membrane. From the endothelial cell, glucose is transported to astrocytes and then on to neurons. This is a multistep process.

It is unclear whether astrocytes feed neurons with glucose or with lactate.

The transport of glucose across the membrane is also a complex process:
The Glut1 transporter recognizes glucose, "swallows" glucose, a conformational change takes place, then glucose is released. Two Glut1 proteins work together as a team (functional dimmer, one is open, one is closed). This process is controlled by energy molecules (ATP).
We do not know anything about these mechanisms in Glut1-DS.

Glut-1 tissue distribution in the body

Glut-1 protein is distributed in the muscle, retina, eye, placenta and heart. Yet we really don't see problems in these areas in Glut-1 DS patients. Is it possible that they are affected over time? We are not sure. There are high levels of Glut-1 protein on retina, yet don't see visual problems.

Pitfalls in obtaining diagnosis
CNS infections
Prolonged seizures
Status epilepticus
Cerebral shunt systems
Some patients with normal glucose uptake have a SLC2A1 mutation.
Can't find mutations in all patients

A lot happens from reading the DNA to creating a functional Glut-1 protein. A defect can occur at any where along the way and cause Glut-1 DS (like in a car factory line)

We know hot spots in the SLC2A1 gene, however patients vary with even the same mutations.
For example: Arg126Cys 9 patients identified
CSF ratios vary between these patients.
Seizure onset age also varies.

Newborn screening would best way to find patients early. However, we need a reliable test that is easily accessible. We don't have a marker yet to do this.

Types of Treatments
Is the 4:1or 3:1 ketogenic diet really necessary?
How about the modified Atkins diet 1:1 (fats:protein/carbohydrates)
How about the low glycemic index diet? We have no experience with this in Glut-1 DS patients.
Does the modified Atkins and the low glycemic index diet really provide enough energy for Glut-1 DS patients? We are not sure.

Alpha lipoic acid at 10mg/kd/day has been used.
It is a co-enzyme in energy metabolism and an antioxidant that normally reduces inflammation.
It may improve cellular glucose uptake but have no data from patients with Glut1-DS.

Diamox 500mg
Is an carboanhydrase inhibitor. It also promotes ion transport across the blood brain barier and modifies the intracellular pH.
In Glut1-DS movement disorders resolved in 1 child when placed on diamox. When used in Germany, 1 child had some benefit, whereas 2 children had no benefit.

Triheptanoin
Triheptanoin is used in the European union as a tracer for butter. It enters the brain like ketones do and replaces energy through replenishing the TCA/Krebs cycle. It does not replace the Ketogenic diet but adds to it.

Animal models
It would be possible to test diets, medications, clinical effects etc. in animals with Glut1-DS.
Mice can be assessed, put into MR-machines and finally sacrificed for cell studies.

Future treatment options
1) **Alternative substrates (ketogenic diet, triheptanoin)**
2) **Stop inhibition (stop using caffeine, phenobarbital, etc)**
3) **promote Translocation of Glucose transporters to the membrane (alpha-lipoic acid ?)**
4) **Activate transporters that are present already-(?)**
5) **Gene transfer-we can't do this yet.**

Focus of European Activities for Glut-1 DS Community
Classification of Glut-1 disease.
International Glut-1DS databank
Next generation sequencing to look for associated genes
Studies in stomatin and Glut1DS

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SLC2A1 Genetic Testing

SLC2A1 Gene
Glut-1 DS is caused by a genetic defect that leads to a protein problem.
The gene is called SLC2A1 (otherwise known as the GLUT1 gene). The SLC2A1 gene has 10 segments of DNA (exons) which contain the information that codes for the SLC2A1 protein. The SLC2A1 protein product (Glut-1) spans the cell membrane. It is a channel for glucose to move from the outside to the inside of cells.

[DNA is within every cell. It is the "recipe" for everything made in the body. DNA is responsible for what you look like and why you look like your parents. DNA is made up of genes or units of information that code for a specific proteins/enzymes/etc. Genes are composed of several different units called exons and introns. The exons are the coding units of DNA. The introns do not code for any proteins, etc.]

Genetic Testing
Genetic testing for SLC2A1 is not perfect. Although testing SLC2A1 will determine if >90% of the common defects are present, there are sections of DNA that are not covered by the available genetic tests. Deletions of large segments of DNA are often not tested for. In addition, DNA outside of the SLC2A1 gene may possibly affect the gene. A negative test result may be due to mutations within the gene that are not covered by the test. In other words, a negative test result means that no mutation was found; it does not always mean there is no mutation.

[DNA is made of individual nucleotides. Like reading letters in a book, the SLC2A1 gene, made of DNA, is "read" to make the Glut-1 protein. Every three nucleotides code for an amino acid. The amino acids are placed together, like cars on a train. The final chain of amino acids is the Glut-1 protein. The exon is the part of the SLC2A1 gene that codes for the protein. Some other parts of the gene are important in this process, while still others are thought to be just extra DNA that has no function.]

Genetic Testing at Children's Medical Center Dallas
Genetic testing began at Children's in 2010. There are stringent state and federal rules for clinical genetic testing. The hospital charge for the test is $2700, but the percent of this charge that is paid and/or covered depends on the specific insurer.

Testing that is available for the SLC2A1 gene
FISH – Fluorescent in situ hybridization
MLPA—multiplex ligation dependent probe amplification
CGH array –comparative genomic hybridization

DNA testing steps at Children's Medical Center.
1) **Sequence the DNA- to look for mutations**
2) **Deletion testing by CGH array-to look for mutations**

Genetic Testing in General
There are 22,000 genes in human genome.
Less than 3000 genes have a known function while the rest have a "theoretical" function.
Genetic disorders are thought to impact about 2% of the U.S. population.

There are 4547 diseases with a known genetic alteration. This number is different than the number of known genes because some diseases may present with different clinical findings, but have defects in the same gene.
70% of hospitalized patients have some genetic component to their disease.

Genetic testing available for large changes in DNA
Karyotype (finds DNA changes [deletions or insertions] of at least 2.5 million bases in size).
CGH micro array (finds DNA changes of at least 5,000 bases in size)
FISH- paints the chromosomes [In different colors so that you can see if pieces are moved to an abnormal location in relation to other chromosomes]

Testing for changes in individual nucleotides
Sanger sequencing- this is the traditional method for DNA testing.
"Next Gen"-new cheaper and faster DNA sequencing method.

Human Genome Project
$3.8 billion was spent to establish the basic sequence of human DNA
[Sequencing the human DNA means that we now know where all of the genes are and what the sequences are. Although we know all of this, we don't know the function of many of the genes]

Moore's Law is a term from the semi-conductor industry that observes a doubling in microchip performance every eighteen months. Similarly, the price of DNA sequencing was decreasing by 50% in the early years after the end of the human genome project. Within the past 5 years, the price of DNA sequencing has decreased faster than Moore's law. DNA sequencing is expected to continue its dramatic decrease in price and be much less expensive in the future.

Exome sequencing
The human exome is the sum total of all of the DNA that encodes the protein (1% of whole genome).
The human exome is 30 million nucleotides. Exome (Next Gen) sequencing is available to run tests on individuals. Clinical exome testing began within the past 18 months. NextGen panel of 22,000 genes including analysis and interpretation is available through Baylor (Houston, TX) for $7000. [Other laboratories are starting to offer exome sequencing at a lower price than Baylor]

In comparison, testing for rare disease genes such as SLC2A1 by traditional Sanger sequencing costs approximately $100-300 per exon. To run a panels of 30 genes by traditional Sanger sequencing costs about $30,0000.

Clinical gene panels- these are useful for "fishing"; that is if you don't know what is wrong with the patient you might want to test them for a host of different genes and see if anything shows up as a likely cause. Although this isn't a bad idea, right now it is better to use targeted genetic testing (traditional Sanger). However this may change in the next 1-2 years.

[These panels can be very useful but one of the concerns about whole exome sequencing is that A LOT of genetic information is produced. Some of the changes might be significant while others are just normal variants that we don't already know about. It can be difficult to interpret the data. Additional concerns include information about other diseases that you are not "looking" for. For example, if the test provided information about late onset disorders such as Alzheimer's, etc. Does the patient really want to know all of this?]

Gene panels are useful and available
For example, Emory University (Atlanta, GA) provides testing for multiple genes associated with Congential muscular dystrophy and X-linked mental retardation.

Other companies and Universities performing 'Next gen' gene panel testing include Ambry Genetics, GeneDx Baylor College of Medicine, and Washington University.

There are no quality standards for many of these panels because they are so new. The SLC2A1 gene is part of the epilepsy panel (GeneDx), thus we will likely see more patients diagnosed with the disease in the future.

Children's Hospital Dallas offers the following tests
Currently the standard genetic test at Children's is Sanger sequencing for single gene defects (e.g., SLC2A1). Within the next 2 years, Children's will begin providing panels of genetic tests by 'next gen' sequencing. By the end of this decade, clinical sequencing will include routine exome and genomic sequencing.

For SLC2A1 testing, alternative to having the test performed at Dallas include Baylor (Houston, TX) and Emory. Commercial labs probably do a good job and may be more flexible with a patient's insurer.

Dr. Toni Pearson
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Movement Disorders in Glut1 Deficiency Syndrome

Movement disorders in Glut-1 DS have been noticed more recently.
1991 "Classical" phenotype of Glut-1 DS was first described in medical literature.
2003 Movement disorders without seizures in Glut-1 DS patients were reported in the literature.
2008 Glut-1 DS first described as cause of paroxysmal exertional dyskinesias (PED).

Outline of Talk
I What are movement disorders?
II Movement symptoms that are seen in Glut-1 DS
- **Persistent**
- **Episodic**

III. Glut-1 DS and "paroxysmal dyskinesias"

How we move is how we interact in the world
Mostly we do not think about how we move in the world. We don't have to think about how we move individual muscles. Many muscles are required for even simple movements; for example, the hand has about 50 muscles. If we want to perofrm a simple action, such as pick up a cup, many parts of the brain must interact with each other for us to perform the movement smoothly and accurately. We can realize how complex this is by trying to build a robot to do a simple human movement.

The brain controls voluntary movement. A few sections of the brain are responsible for this:
1)Motor cortex
2)Basal ganglia- selects patterns of movement , which muscles move and when, involuntary movements
3)Cerebellum – responsible for balance, coordination, and timing of movements.

When these areas of the brain are compromised, then movement disorders result. For example: alcohol causes temporary dysfunction in the cerebellum, causing slurred speech, poor balance, and incoordination.

Categories of movements
Types of movement disorders include:
1) Involuntary movements
- **Dystonia- slow sustained**
- **Quick and jerky movements such as chorea myoclonus**

2) Balance and coordination
3) Abnormalities of tone too stiff or too loose

The 3 most common movement disorders in general and in Glut-1 DS are:
1)Dystonia-involuntary postures, sustained
2)Chorea-quick, random appearing, "fidgety" movement , or dancing-like movements
3)Ataxia- missing the target("intention tremor") on finger nose test or wide based, unsteady gait

Glut-1 DS movement disorders
Glut-1 DS can cause motor symptoms that are there all the time or that come and go.
The most common persistent symptoms are:
Abnormal gait 90%

Dystonia 86%
Chorea 75%
Intention tremor 70%
Myoclous 16%

Most patients have more than 1 motor symptom at the same time.

<u>There are episodic non-epileptic events in 28% of Glut-1 patients, such as:</u>
Unsteady gait
Weakness
Dystonia
Choreoathetosis
Irritability

<u>Triggers for these events can include:</u>
Fasting-before meals
Exertion
Fatigue
Lapse in ketogenic diet
Sometimes the trigger is not obvious.

<u>Paroxysmal exertional dyskinesias (PED)</u>
Paroxysmal means episodic
Exertional means with exertion
Dyskinesia means involuntary movements

PED was first described as a type of episodic movement disorder in the 1970's
The onset of PED is almost always in childhood.
Episodes typically last between 5 and 30 minutes; may last up to several hours infrequently.
Familial and sporadic (not seen in other members of the family) cases are reported in the literature.
There is often a variety of different movements.

Multiple families with with PED's due to SLC2A1 (GLUT1) gene mutations were reported in 2008.
In one of those families, family members had both PED and anemia.
Iin another study, 10 patients with PED (and no other affected family members) were tested and Glut-1 mutations were found in 2 of 10 patients of those patients.
Glut-1 DS is the only known cause of PED at this time.

In 2011, Glut-1 mutations were associated with another disorder causing paroxysmal dyskinesias, known as "paroxysmal choreoathetosis and spasticity" that was initially labeled and classified as a dystonia disorder called DYT9; It is now known to be caused by Glut-1 DS.

<u>Summary</u>
Glut1 DS is associated with persistent and episodic movement disorders.
Glut-1 DS is very likely under-diagnosed in patients who don't have seizures.
An episodic movement disorder may be the prominent feature when epilepsy is absent.

A lot of children are diagnosed with cerebral palsy who may have developmental disability and abnormal movements. Some of these children may actually have Glut-1 DS.
Physicians should think about Glut-1DS when trying to diagnose patients with movement disorders.

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Glucose transporter type I deficiency syndrome: 2011-2012 review and 2012-2013 agenda

I.2011-2012 Review
We have a large laboratory of dedicated staff (neuropsychologists, genome diagnosis, biochemistry, MR spectroscopy, etc.)

Scope of practice
Testing
Understanding
Treating

Our Approach
1) **Hypothesis driven: based on patient observation, create a hypothesis, develop a research plan, develop methods, accept or reject the hypothesis.**
2) **Need-driven: identify an unmet need or unexplored opportunity, assess resources and new knowledge needed, research and development, fulfill or modify the need.**

Lines of Research
A. **G1D**
1) **Patients**
2) **Mice**
3) **Therapy**
4) **Diagnosis**
5) **Patient registry**

B. **Pyruvate dehydrogenase deficiency**
C. **Human brain metabolism**
D. **Human muscle metabolism**
E. **Clinical investigation of mitochondrial disease**

Main events
The laboratory moved to a larger facility.
We established a rodent core facility with video monitoring for rodent seizures.
UT Southwestern initiates a rare diseases in 6 year plan. Pascual lab is second in terms of focus for plan.
Bruce Beutler, MD, UT Southwestern receives the Nobel Prize (fifth Nobel laureate at Southestern)
Triheptanoin (C7) clinical study starts for G1D patients.

Funding
2 RO1 NIH grants funded
U10 NIH Neuronext funded

Others

We had 11 Publications 2011-2012
1) Brain tumor and human brain metabolism
2) Human brain metabolism
3) G1D mouse: metabolism and epilepsy

II.Glucose Metabolism
Glucose is eventually turned into ATP (energy)

Glucose metabolism generates:
1) Energy
2) Neurotransmitters
3) Energy and neurotransmitters

Our new finding related to glucose metabolism is that there is no loss of TCA/Krebs cycle function.
[The TCA/Krebs cycle is important for metabolizing glucose]
It isn't the total amount of energy(ATP) that is the problem in G1D, the problem is in neurotransmission.
The molecules need to be made and stored.

The PET scan (Positron Emission Tomography) is a test that we can use to evaluate brain glucose metabolism. Some of the sections of the brain take up very little glucose and others more. The question is; can we manipulate the brain regions to improve function?

III.Current project in our laboratory
The new MRI-compatible EEG cap picks up more seizures that a regular EEG. It picks up things that you don't even see by watching the child.
How can the whole brain be seizing yet the child still communicates to you? This doesn't seem to make sense if seizures fully impair brain function.
We conclude that the EEG is just a limited way to evaluate brain activity.

The Functional MRI (FMRI) is useful in evaluating brain seizure activity.
New technique to follow a seizure using Functional MRI.
In the FMRI, we see that only parts of the brain are seizing. It seems to be a highly localized problem.
Additionally, seizures seem to inactivate other brain parts.

Mice are used to Evaluate Some Theories
Glut-1 Mouse with catheter into vein in neck [allows access to the blood vessel]
Mice are sacrificed to study the brain.
We can look at the electrical activity of the brain in the lab.
A normal mouse that does not have G1D has no extra activity.
The G1D mouse has a wave of electricity in whole brain all the time.
If we remove thalamus, then this goes away.
The thalamus is a part of the brain that is a signaler.
The thalamus and the brain cortex have the lowest amount of Glut-1 protein quantity.
Why is Glut-1 protein not reduced equally in all brain cells?
Glut-1 protein is heavily regulated by other things in the brain.
However, the thalamus and the cortex are the worst at compensating for a reduction in Glut-1 protein.

We can inject glucose into brain and count the glucose molecules in brain. In specific we might want to count the cortex, thalamus, and the striatum.

A tiny amount of glucose in an area could be sufficient whereas another area needs more glucose to function properly.

The krebs cycle metabolites seems to be roughly normal.
The problem is in acetyl CoA, which is low in G1D.
Why is acetyl Co-A low but other downstream metabolites are nomal?
Because mice can find a way to increase ketone metabolism by mobilizing fatty acids which in turn produce the downstream metabolites.

We know from our research that there is nothing wrong with cells in growth, size, etc. in the brain in G1D.
Cell to cell communications in brain, however, are problematic; these are synapse problems.

[A synapse is the junction between nerves in the brain. It is the way that nerves "talk" to each other and relay signals. Synapses rely on various chemical signals to transfer a message from one neuron to another.]

The strength of synapses in G1D are weaker than in controls (terminates ability to excite). There is not enough excitation and inhibition is way down.
Excitation doesn't change much; what changes is inhibition is way down.

The brain's "pacemaker" seems to be located somewhere within the thalamic - cortical organization.
Is there a pacemaker?
We evaluated the thalamus of the mouse and found electrical currents. In G1D we see short bursts of activity. This maybe indicates that the thalamus is the pacemaker of the brain in G1D seizures.

In theory, if you identify the mechanism (the pacemaker) and know how the cell is working it might be possible to mediate the pacemaker through other molecules. Inherent in this is a possible chance at a therapy. Maybe we could just fix the synapse? This would open up the field of synapse function in neurology to areas that were not previously known.

Need to look at brain metabolism when the animal is alive and awake in order to think about new potential treatments that may work or not work.

<u>NMR or MRS technology</u>
NMR or MRS technology allows a new view of patient and mouse brain metabolism.
A normal mouse brain is infused with radiolabeled glucose and we evaluate which chemicals are produced.
We evaluate which chemicals are labeled with the radio tag.

The neurotransmitter production mechanisms can be evaluated.
[A neurotransmitter is a chemical on the synapses that is the actual signal between neurons.]

<u>Triheptanoin</u>
Triheptanoin is a triglyceride. It is a 7 carbon molecule.
It is like MCT oil which is a mixture of many triglycerides, but which does not contain C7.

It is picked up by liver and makes ketone bodies. Not the usual ketones, but beta keto pentanoate (an odd-carbon ketone body not measured in clinical laboratories.
C7 refills the Krebs cycle. The ketone bodies are taken up in the brain.
We weren't sure if the triheptanoin was taken up directly into the brain.
Research was done in the mouse model. First we gave triheptanoin and then we saw that it produced acetyl Co A and glutamine in the G1D mouse better than in control mice.

Although you can increase acetyl Co A and glutamine; the question is "Does it fix the seizures? "
Yes, it seems to fix the inhibitory problems in G1D. This is a work in progress on a 5 year NIH grant.

<u>G1D patients coming in for the C7 Triheptanoin</u>

1) These patients can not be on ketogenic diet as the C7 may break the ketosis. This is the situation for now, until current research on the compatibility of C7 and the ketogenic diet is completed.
2) DNA mutation must proven.
3) Must be able to travel to Dallas 3 times

<u>Procedures in the C7 trial.</u>
Medical examination brain metabolism MRS and MRI (5-10 minutes),
Functional MRI
Patient wears an EEG cap in MRI
Neuropsychological study (45 min)
Blood sample analysis
Start treatment with C7
Repeat assessments

Although we need to first prove whether C7 works for G1D, it is possible that, in the future, we could: 1) add it to the ketogenic diet or 2) replace the ketogenic diet.

We are accumulating the data to answer these questions and for use in future clinical trials

<u>Genetic Testing</u>
We have DNA testing for families
We have a created a public informational resource with definitions, inheritance patterns, and treatment options: http://childbrainfoundation.org/g1d.html

Question and Answer Session with Dr.'s De Vivo, Pascual, Klepper, and Park

1) **"When the ketogenic diet does not work, has there been any other study regarding the MCT transporter?"**

   There are mice with MCT deficiency yet no condition has been identified in humans.

   Ketone bodies were thought to be a metabolic waste product until late 1960's when it was shown that the brain could use ketones as energy. The use of ketones is an auxiliary system. When the body gets glucose it won't use ketones. MCT carries ketones into the brain if the patient is fasted. Ketotic hypoglycemia can occur if patient is on a ketogenic diet and MCT doesn't work.

   All patients respond to the ketogenic diet, but not completely in some cases.

2) **"Are ketones in place of the glucose molecules? Why use alpha lipoic acid (ALA) which helps glucose?"**

   Glucose isn't zero when the patient is on the ketogenic diet. We always need some glucose to keep the citric acid cycle running. We don't know if ALA does anything to the glucose transporter. In the presence of high concentrations of ALA we can correct the Glut-1 defect in the laboratory. However, there is no clinical evidence that it works. 50% of the Glut-1 protein is generally available to the patient with Glut-1 DS. Both glucose and ketones are present and we want them both to work optimally.

3) **"What is the clinical correlation between the value of ketone measurements and clinical usefulness? Restatement- What is the evidence that the diet is beneficial? "**

   The evidence for a given concentration for ketonemia is not known. How high can your ketone levels go up and how fast is an issue. Infants are 5-6mmol/L generally, some patients can get higher 7-8 mmol/L. The question isn't whether you are going to replace the glucose but rather add something else to it. There are no other fuels for brain energy. The goal is to achieve the maximum of ketones to brain. If you get more than what you need it probably isn't a problem. Don't check ketones in the urine as they are not accurate. Restate question- Is 4 mmol/l better than 3mmol/l? We can not determine this right away, we can only evaluate over the long term. Rather be safe and have a higher ketosis.

4) **"Would raising blood glucose improve glut-1 DS?"**

   Not really, this would create more problems as the body wants to maintain correct blood glucose. It is possible to give more meals and higher glucose to maintain a glucose level at around 100. This would not increase the lumen glut-1 protein levels. If Glut-1 DS patients have a glucose tolerance test (drink glucose) they improve as you raise their blood sugar. Their EEG improves, after 2-3 hours the glucose drops and the EEG worsens. Patients received diazoxide to maintain blood sugar in an elevated state and a down regulation of glucose transporters was noted. If blood sugars are reduced the number of glut-1 proteins increased. Patients taking diazoxide can worsen. The short term benefit of increasing blood sugar works, while long term blood glucose elevation worsens.

5) **"Glut-1 symptoms seem to follow puberty cycles, how does puberty affects Glut-1 DS symptoms?"**

   Puberty is complicated and we don't know a lot but neurological function is affected by sex hormones.

6) **"Is there evidence for structural change that is irreversible? I.e if there were a cure would there still be damage?"**

   No evidence for irreversible change. This is an advantage. The MRI's are normal and we have looked very hard (cell death, size, numbers, etc).

1) **"Some children's symptoms increase at rest or with anxiety? Is there something we can do?"**

It could be a behavioral problem that isn't associated with Glut-1 DS. Maybe give him more carbohydrates for a short time to see if there is a relationship. Group poll: anxiety and nervousness can affect child. The stressed brain has a certain metabolic profile before and after starting the keto diet. The stress profile is lower on ketogenic diet. We could measure in MRI/ MRS but what does it tell you? The need for the registry is to help us identify what symptoms patients are having. Paroxysmal phenomena are common in Glut-1 DS. Running- rest—later paroxysmal events. Probably things are going on throughout the whole cycle. Neurological symptoms are affected by many things such as stress, heat, etc. These things can cause dysfunction in nervous system. We just don't understand why this happens.

2) **"Does the brain need less energy as we age?"**

PET scanning is the best group of work done that addresses this issue. Metabolic brain needs are low at birth and the zenith is at 3 yrs to 10 years then lowers at about 16 years then lowers again as people age. This is why the ketogenic diet should be aggressively used until at least 10 years of age. Maybe a ketogenic diet in the first 10 years, then a modified atkins in the second decade might be useful, this is a thought. What about the transporters? MRIs performed to evaluate oxygen consumption (brain used oxygen for metabolism) show a 20-23% in decrease in brain oxygen consumption in Glut-1 patients. In dementia the same tests are 5% loss. If we give carbohydrates, it near normalizes but is transient.

3) **"Can fine and gross motor skills improve with PT? How soon should you see the results?"**

No easy answer because no one systematically evaluated this. The benefits of PT may be over generalized. We do see improvements with the ketogenic diet. It is difficult to assess the impact of the ketogenic diet on cognitive function. Again we would need to evaluate the child before and post the diet. Klepper has done this with his patients but there is such a large amount of data that it is difficult to evaluate. There are a number of issues that might be important to study, but we are not going to study all things. As long as things make sense we will do it. We won't take someone off of the diet to determine if it works.

4) **"Amyloid beta precursor protein- protects from Alzheimers- does this have anything to do with Glut-1 DS?"**

Many genes function in different ways- sometimes genes prevent you from developing too greatly. Some genes are gatekeepers. We don't know if there is another gene that when getting rid of will help Glut-1 DS. There is a camp of Glut-1 scientists who are learning about the Glut-1 gene because if it is deregulated it will deregulate cancer. Cancers tend to over-express Glut-1 DS. Some children are born with vascular malformations; however if have Glut-1 DS the frequency of this issue is less. There are some benefits with Glut-1 DS in downstream therapeutic targets.

5) **"Funding research- where do doctors get funding for research? "**

Funding comes from National Institutes of Health (associated with indirect costs), non-federal sources such as various philanthropic foundations.

6) **"If the blood ketone levels are greater than 4-5mmol/L is that a good thing? "**

There isn't much use for ketones over 6mmol/L because the blood will become acidodic. The kids will also lose energy and appetite.

7) **"What do we know about early diagnosis than later diagnosis?"**

The data haven't been looked at yet. The natural history data needs to be evaluated more thoroughly. In Alzheimers we see reduced Glut-1 activity but as a consequence of the disease, not as a precursor. In the oldest patients of Glut-1 DS we have evaluated we do not see any evidence of early onset dementia. In the mouse model, 2 yr old (old) mouse we see no evidence of Alzheimers. We do not think that Alzheimers is a long term concern. What is the expectation of rescuing the phenotype when you intervene early versus late? We don't have the data for this. We don't have a uniform way of managing the patients. i.e. patients diagnosed at various ages, patients treated with more or less rigorously with the diet. Not all patients are compliant with the diet. Additionally, everyone is somewhat different and there aremany different mutations. Intuitivey we think that starting earlier is better. We can not have negative controls-i.e. it is unethical to withhold treatment from a child to see how they do.