2019 Glut1 Deficiency Foundation

Conference Summary Report

much gratitude to Chantal Sanchez, Kris Engelstad, and all the speakers for their help in preparing this summary

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Glucose Transporter Type 1 Deficiency Syndrome is regularly referenced using a variety of terms, and these presentations were no exception. In the interest of clarity and uniformity, we have used the term Glut1 Deficiency throughout the summary. Glucose Transporter Type 1 Deficiency Syndrome is also known as: Glut1 Deficiency, Glut1 DS, G1D, Glut1, Glut1D, and De Vivo Disease

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Glut1 Deficiency: Past, Present, and Future

Glut1 Deficiency as a rare disease

Glut1 Deficiency is what we call a rare disease. A rare disease is defined as a condition that affects less than 200,000 citizens in the United States. That definition largely applies around the world but it is related to the population of the country or the region so for the United States, it's a condition affecting less than 200,000 people.

We have about 10,000 human diseases that we are aware of and of those, there are at least 7,000 that can be defined as a rare disease. The aggregate of all of those rare diseases affect about 30 million citizens in the United States. So about 10% of our population is affected by one or another rare disease and unfortunately that affects mainly children (2/3 of rare diseases affect children). At least 80% of them are genetically determined, usually due to a gene, such as the SLC2A1 gene—the Glut1 gene.

The issue that we need to address is that only 350 of those 7,000 rare diseases have a disease modifying treatment that has been approved by the FDA. That's only 5% of all of those diseases. A lot has been done but there is a lot more that needs to be done as well. Glut1 Deficiency is really the quintessential example of a rare disease. We have moved through an increasing understanding of the complex clinical presentation as there is phenotypic diversity and it requires the talents of a lot of subspecialists and other professionals in order to provide optimal care for patients with Glut1 Deficiency. In 1991 the clinical phenotype was first described and we have now come to a point where we are dealing with conditions that look very much like Glut1 Deficiency but they don't seem to have any mutation in the Glut1 gene, so there is some other genetic mechanism affecting the Glut1 gene, maybe indirectly as a target of the unidentified gene and so we have coined the term Glut1 Deficiency Syndrome-like disease.

There is a gene called the PURA gene that has been identified that produces a low glucose in the spinal fluid (hypoglycorrhachia) and some of the same clinical features that we see with Glut1 Deficiency, but its due to some other genetic mechanism, and we now have to add that to our list of things to study to increase our understanding of how hypoglycorrhachia and the clinical phenotype can emerge in the setting of a genetic defect unrelated directly to the Glut1 gene.

Past

The story began with two infants who presented with two important biomarkers: low glucose and low lactic acid in the spinal fluid. A red blood cell uptake assay was developed knowing at the time that the transporter for glucose in the red blood cell was in fact identical to the transporter for glucose in the brain, across the blood brain barrier, and into the different brain

cells. We had the distinct advantage at the beginning of developing a functional assay for the condition before they knew what the genetic cause of the condition was. Seven years later, the mutated gene causing the Glut1 Deficiency phenotype was found – the SLC2A1 gene.

At the time, it was known that the brain could either use glucose or ketone bodies— those are the only fuels available to the brain to be used to meet the energy demands of the brain. If you weren't getting enough glucose into the brain, perhaps if you supplemented with ketone bodies, that might be helpful, and that has continued to be the standard of care to the present time as a symptomatic treatment for this condition.

Red blood cell uptake assay

Glucose goes into the cell and gets converted into pyruvic acid. Pyruvic acid is in equilibrium with lactic acid. If not enough glucose is getting into the cell, then understandably you're not making enough pyruvic acid and lactic acid. When you do a spinal tap and measure glucose and lactate, you'll find that it is low as a result of the fact that the blood glucose isn't properly being transported into the brain under those circumstances.

We developed a red blood cell uptake assay and did it with Joey, the first Glut1 Deficiency patient at that time and found that his uptake by the red blood cells was in fact low. Dr. Klepper joined a few years later and refined the assay and published on it. It is still considered the gold standard as the functional measure of the genetic defect.

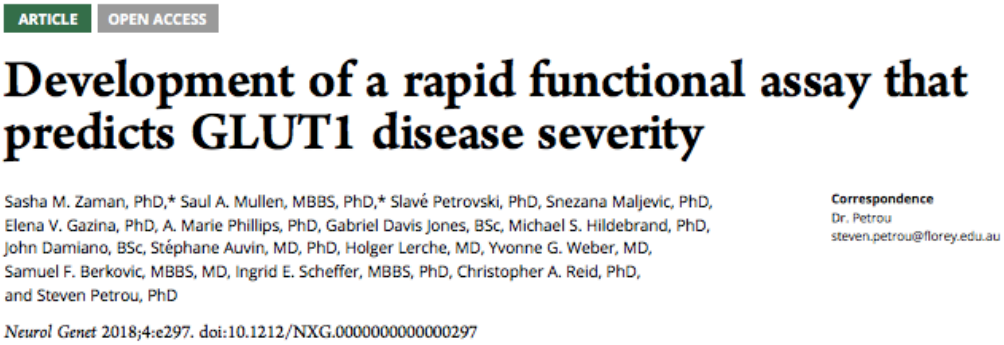
In the next 150 patients that were seen, the CSF glucose and lactate were measured and in every case, the values were low. About 90% had a value between 40mg/dL and 20 mg/dL, which is distinctly low. And every patient had a value less that 60 mg/dL. This has continued to be a very important marker of the possibility that a patient has Glut1 Deficiency. The assay was developed in the laboratory at Columbia and it has turned out to be a very useful assay. The difficulty with it is that it is labor intensive, demanding in the technical sense, and expensive.

If you correlate the clinical severity of the patient with the degree of reduction in the uptake of glucose by the red blood cell, there's a very good correlation. There is also not much margin of error here, so if you're born with 100% that's fine, if you're born with 80% that's okay but if you're born with 75% you are increasingly symptomatic. If you go below 25% that is essentially embryonically lethal. What this means is that you need Glut1 and a little reduction is okay but more than 25% is inadequate to meet the needs of the brain particularly during infancy and childhood when the metabolic rate is very high.

There are other assays that are evolving such as a functional assay from Metafora that measures the presence of the Glut1 protein on the surface of the red blood cell using some very elegant techniques. From their paper in 2017, they showed that in a group of 30 patients, 7 of them fell in the normal range but 23 fell below the normal range. It seems to be a rather robust assay and can be done fairly quickly and inexpensively. It is a tool that is now available for the physician who might first encounter the infant who might have had a seizure or some funny eye movements at 2 months or 6 months of age and the question would be, does this

child have Glut1 Deficiency. We'll continue moving back hopefully to the newborn screening period where we ultimately need to be, particularly as gene therapy is on the horizon as a real possibility as a disease modifying treatment.

There is an additional test which takes frog eggs, Xenopus oocytes, and they are engineered in such a way that you recreate the mutation that the patient has causing their Glut1 Deficiency syndrome, you inject the cRNA into the frog egg and look at the capacity of the protein being made by that gene in the frog egg to transport glucose across the membrane of the Xenopus oocyte. What this showed was that if you have a severe phenotype, you can correlate it with the severe pathogenic mutation and if you have a milder clinical phenotype, it correlates with a mild pathogenic mutation. The severity of it is measured by the velocity at which glucose can be transported from outside the cell to inside the cell, so there is an increasing array of diagnostic tools that we have now in order to validate the clinical impression the patient has with Glut1 Deficiency.



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Development of a rapid functional assay that predicts GLUT1 disease severity

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One can also look now at the signature that one sees on the PET scan and there is global reduction in the uptake of glucose into the brain but there is also regional vulnerability so some areas are more vulnerable to Glut1 Deficiency than the rest of the brain: temporal lobe, thalamic area, and the cerebellar circuits. This is another diagnostic capacity that is available to confirm any clinical suspicions that the patient has this condition.

A while ago, we took 109 consecutive patients who had been referred to us with the possibility that the patient might have Glut1 Deficiency and this was published in 2011. They all had a clinical picture that you might consider to be consistent with Glut1 Deficiency. They all had CSF hypoglycorrhachia and hypolactoracchia so you had those biomarkers present in the clinical setting. We did the red blood cell glucose uptake assay and what we found was that about 2/3 of the patients had a decreased uptake and about 1/3 of the patients had a normal uptake. We then analyzed that tissue in greater detail to see whether there was a disease-causing mutation in the Glut1 gene. And we found that in almost 100% of the 74 patients, there was a disease-causing mutation, proving a very good correlation between the red blood cell uptake assay and the presence of a disease-causing mutation. In the 35 patients we found only one of the 35 who had a disease-causing mutation but it was what we call a kinetic mutation. The assay is run at 4ºC and at 4ºC that mutation functions normally. But at your body temperature of 37ºC it

functions abnormally and causes Glut1 Deficiency. It is therefore a false negative in the setting of this particular assay.

Glut1 Deficiency Syndrome and Erythrocyte Glucose Uptake Assay

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About 2/3 of the patients who have a clinical picture consistent with the disease—a low CSF glucose and a low CSF lactate— will likely have a disease-causing mutation in the Glut1 gene. Alternatively, we have about 1/3 who do not reveal a mutation in the gene and we call that hypoglycorrhachia not otherwise specified or Glut1 Deficiency syndrome-like phenotype. Kris Engelstad and others have analyzed those 34 patients and others and they break up basically into two groups:

Transient symptomatic hypoglycemia of infancy: transiently they have a low CSF glucose and some associated symptoms but as they work their way through infancy, everything gets better and they end up developing fairly normally and roughly half of those 34 patients fell into that category.

Persistent symptomatic hypoglycorrhachia of infancy: here the outcome is guarded.
Both groups look alike in the sense that the CSF glucose and the CSF lactate are low, the red blood cell uptake assay is normal, the Glut1 gene analysis is negative and we found 1 patient out of this group who had a PURA mutation.

Now we have to understand why patients with the PURA gene mutation have hypoglycorrhachia and they have a perfectly normal Glut1 gene and that work is going on now.

We followed a 7-year-old child who was quite severely disabled. We had 4 lumbar punctures and the CSF glucose was in the 20's and 30's, the CSF lactate was low and the red blood cell uptake assay was normal. This is a complex phenotype we have come to understand and we have shown this in different generations.

Neurological Domains affected in Glut1 Deficiency:
Cognition: intellectual disability, a cognitive phenotype, persists with the patients throughout the life cycle
Behavior: epilepsy, attention, distractibility, impulsivity
Movement: spasticity, ataxia, dystonia
3 major pathways that are affected here:

Pyramidal Tract: motor system

Cerebellar System: balancing system
Extrapyramidal System: system that modulates posture and control and the quality and fluidity of your movement

You have varying degrees of these symptoms as you see in each of the patients from very mild to rather substantial and if you have all three elements represented you have what people now call the classical phenotype which represents roughly 80%- 90% of the patients.

Increasingly, since we have learned about this problem we have to know more and more about the evolution of the condition through early and late childhood into adolescence and then into adulthood.

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Long-Term Clinical Course of Glut1 Deficiency Syndrome

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A paper was put together which profiled 13 patients that have been followed for 20 or 30 years and what you can see is that early on you have evidence of developmental delay, ataxia, deceleration of head growth with or without the acquisition of microcephaly, the onset of seizures in in infancy (the epileptic phenotype is an infantile disturbance and later a series of different kinds of movement disorders dominated principally by dystonia). This is the emerging understanding of Glut1 Deficiency as it evolves over time from infancy to childhood to adolescence and adulthood.

How does this first present?
At the beginning, this is a condition that presents episodically. It's a paroxysmal disorder in an otherwise normally developing young infant and then increasingly you see that its more of a persistent problem with punctuated paroxysmal events. We find seizures and a peculiar eye-head movement abnormality to occur, for the moment, only in the setting of Glut1 Deficiency.

How do we manage this problem?
Early diagnosis and treatment are so important for these genetically determined diseases that affect the developing nervous system. Ultimately, newborn screening is where we want to be. The ketogenic diet remains the standard of care. Despite all of its complexities, difficulties, and compliance issues, it still is providing the brain the only alternative fuel for metabolism, mainly ketone bodies. We like to measure blood ketones and tend to discourage people from measuring urine ketones because it is not quantitative and the goal is to know what your blood beta hydroxybutyrate level is to know whether you are doing a good job with the diet. We need

to understand that this is a complex phenotype and we need to take a multidisciplinary approach with the management of these patients.

What does the future hold?
Breastfeeding, I believe, is neuroprotective. Nature designed breastmilk to be high in fat and we know that there is a delay before the onset of this condition and often times, I have met with families where the mother breastfed for a longer period of time and the child seemingly has benefitted from exposure to breastmilk for a longer period of time as opposed to a shorter period of time. We have to do studies that will ultimately prove this.

We know the ketogenic diet is the standard of care and we like to think that if you start it as early the outcome will be better than if you start it later but again, we have to prove that. This is complicated because every patient is different, every patient has a different kind of mutation, which has its own degree of pathogenicity and poses a different degree of phenotypic severity in the patient, so we haven't been able to tease that out to everyone's satisfaction.

We are increasingly aware of the milder phenotypes. There may be some patients that have CSF values that are low, so we have to continue to explore that and now with precision medicine and increase use of gene panels for patients with epilepsy and movement disorders we will be picking up on more and more patients who are presenting in ways that don't immediately remind us of Glut1 Deficiency.

Newborn screening is essential in this kind of situation and what you need is a high throughput screening technique so that we can identify the genetic mutation in a large number of Glut1 Deficiency babies. We have 4 million babies a year born in the United States, we have 125 million babies born in the world, you would have to have an assay that allows you to quickly look at that number of people and give you an answer within 24-72 hours as to whether there is a problem or not.

And then we have to develop more disease modifying treatments. We only have to affect a small improvement in the genetic influence in making the Glut1 protein. So maybe a small molecule approach for the normal gene, the normal allele or alternatively, repairing the genetic mutation by gene therapy as has been done effectively in model mice.

Dr. Monani and others at Columbia reported a very nice paper in Nature Communications in 2017 with the mouse model we developed in 2006 and we showed early treatment with gene therapy provides a better outcome in the mouse model than later treatment. Glut1 Deficiency newborn screening will permit us to take a proactive treatment of the pre-symptomatic patient.

ARTICLE

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Brain microvasculature defects and Glut1 deficiency syndrome averted by early repletion of the glucose transporter-1 protein

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There are advances going on currently collaboratively with Dr. Smitha Jagadish in an effort to develop an effective gene therapy for Glut1 Deficiency Syndrome and there are major hurdles that one has to overcome to solve this problem and create an effective treatment:

1. Engineer the so-called capsid so that the construct goes to the cell you want it to go to, not just indiscriminately any cell or to the wrong cell. You want to target the cell you want it to go to and that requires sophisticated engineering of the capsid. The gene is put inside of a viral vector and that viral vector has to be directed at the cell of interest.
2. Optimize the promoter so you make the proper amount of the protein
3. Manufacturing it so that you have enough available to treat a human not just in the animal model but many humans

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Registry and Research

A fundamental fact is that there isn't very much known about Glut1 Deficiency and what it does to the brain. This has implications for the treatment. You are very much challenged to be able to treat the condition if you don't know what is happening to the brain.

What we know about this disease
Glut1 Deficiency is a regional disease in the brain. Although when you take an EEG you see that the seizures are coming from all over the brain, if you run an MRI and look at not just size and shape but also activity, the problem seems to be localized to very selective brain regions. Therefore, it's not just a global brain problem but also a highly localized problem.

The idea was to look for certain cell types that are more problematic than others and that has a lot of implications because if you have to treat every single cell in the brain, that is a tall order. If you are dealing with one or a few cell types, that's a different concept in terms of managing the problem. We have taken advantage of the Glut1 mouse model and with an electrode recorded electrical activity coming from a single neuron which allows you to identify what electrical currents are happening with that neuron and that's important because neurons communicate through electrical currents.

Very early on, we found in the lab that there is a brain region that is very prone to have very high abnormal activity and we call that the thalamus (thalamic oscillations). When you stimulate the brain with current and look at the electricity going through that part of the brain you can see that in the Glut1 Deficiency brain there are highly abnormal spikes that go on continuously. And that's the way the brain works, a tiny bit of electrical activity going in somewhere and then the brain exhibits this activity. In Glut1 Deficiency you have all of these abnormal spikes that perpetuate that phenomenon for quite a while.

How does this happen?
Dr. Karthik Rajasekaran spent years looking at different cell types and identified the one cell type that is doing this. In the neuronal electrical activity, the spikes represent synaptic activity, they are synapses that allow cells to communicate and every little spike is a communication event. In Glut1 Deficiency, this is highly diminished in that particular cell type. The good news is that this doesn't happen in any of the other cell types. It is only that one cell type that doesn't fire the way it should be firing.

There are many ways to achieve excitation in the brain but there only one fundamental way to achieve inhibition. This is a failure of inhibitory cells to work the way they should. It is a

problem of impaired inhibition with normal excitation. Therefore, you have this prolonged, repetitive, ongoing activity that should have been terminated.

Dr. Vikram Jakkamsetti went ahead and applied that concept to a different disorder downstream Glut1. There is one enzyme called pyruvate dehydrogenase, and what he has been doing is have an animal respond to a whisker stimulation. You can touch it, you can stimulate it electrically, and mice are very sensitive to whisker stimulation because they don't see very well. They rely on their whiskers to feel the environment and when that happens, in the brain there is a very precise amount of electrical activity that has to be taking place so the animal knows how to orient itself. What he found was that in a normal animal, there is a very compact electrical response in the brain whereas in mice with the disorder, the responses are all over the place, they are much broader over time. They are not as tight as they should be and so the animal is not getting a precise and limited amount of information from that whisker stimulation. Instead it is getting a very broad and hazy type of activity. It is important to note that this is a live animal behaving as it should, it is not a cell in a test tube. It is a very different from working on an isolated piece of brain or cell in terms of the impact.

Dr. Karthik Rajasekaran went ahead and experimented treating these cells with different drugs. He looked at the amount of the compound *Pilocarpine*, a toxin, you have to inject in the mouse order to induce a large seizure. When you give a particular amount of that drug, you get with that amount of drug injected, 75% of the mice have a life-threatening seizure and it is much easier in a mouse that has Glut1 Deficiency than in a mouse that does not. He also used in mice a ketone body byproduct of C7 oil. It's an unusual ketone body, not your typical ketones that are made from the ketogenic diet but the ketones that are made from the C7 oil (they are different) and there is no induction of seizures in these mice. So that was the basis for doing the trial. Then he moved on to the many different drugs that are used to treat Glut1 Deficiency: anticonvulsants, seizure drugs to see which one would have an impact, no impact, or make it worse.

Richard Wang had the idea of giving patients a transfusion. Capillaries, very tiny blood vessels, deliver glucose to the brain. What happens in the body- not in the brain but in the rest of the body- is that glucose has to go from either inside the red blood cell or inside the capillary lumen, through the wall and into the organ. However, in the brain it is different. The blood vessels are packed. They don't leave any room for the fluid. They are totally packed together so the flow of glucose really doesn't happen from the fluid or from the red blood cell into the wall, it actually happens primarily outside of the red blood cell not so much from just the fluid. In other organs, it would go from the wall of the blood vessel but in the brain, you would have to go through the wall of the blood vessel and through the wall of the red blood cell— two steps as opposed to one. You can imagine that in Glut1 Deficiency where it could be operating at 50%, having to go through two filters results in a lot less flow than the other organs so the idea is can we eliminate one of the filters.

The idea is to replace the blood in a person and to be able to supply a full amount of red blood cells with a full amount of Glut1. A transfusion will last 120 days and there are conditions in

which people get transfused through life. We know the safety profile and logistics of doing such things from other fields of medicine and we are hoping to begin soon. We will be looking very carefully at the whole picture to see the impact that is made and if its meaningful for some people we will share that.

Drug Discovery
Drugs which increase Glut1 mediated transport of glucose in cell assays will be applicable to clinical improvement in Glut1 Deficiency.

Jason Park, MD, PhD has the idea of setting up robots that would read Glut1 activity in cells and screen potential compounds and check for increases in Glut1 activity in those cells. Unlike mouse work, this involves tens of thousands of compounds to see which one of them may do something, some of which are very well-known drugs, some of which are not. We are going to go ahead and initially look at 1,100 drugs that are approved by the FDA for other diseases so if you find that one of them works, all of the regulatory safety work is already done so this project is in the initial phase.

Bruce Posner, PhD has a lot of experience doing this, particularly in cancer, and has been able to license 10 new drugs with pharmaceutical companies using this procedure. The advantage is that these are relatively simple things to do when we're talking thousands of drugs being screened one at a time because it's a robotic project. We have a cancer cell line that has a lot of Glut1 so it is very easy to work with in terms of looking at changes in Glut1 activity in these cells as opposed to the brain cells which do not have a lot of Glut1 when trying these different drugs. In the assay, there will be 360 wells in a plate each of which has a number of cells and the robot applies one drug at a time in each well and there will be glucose that we made fluorescent and if you have more glucose, there will be more fluorescence.

Initially you have a chemical library in which you typically find 2,000-3,000 drugs that increase Glut1 function. Many of them will be toxic to a person or will be doing other things also so you have not discovered at that moment 3,000 new drugs to treat Glut1 Deficiency. You have potential leads that will increase Glut1 but whether they can be given to a human being is a different subject. So you have to run a number of confirmatory assays and normally you narrow down the group of them to a couple of hundred candidates and then you do a great variety of other tests to comprehend the mechanism and prove that this is effective and if you are fortunate, you will have a lead compound. You will still have to find dosage and prove toxicity for Glut1 Deficiency even if you are using a well-known drug for a different disease. If you don't have that information, you have to generate all of that knowledge through phase 1 clinical trials.

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Consensus Work and Global Updates

What has happened in the world of experts meeting for Glut1 Deficiency and the issues involved:

- Glut1 Deficiency Foundation Conferences
- Global Ketogenic Diet Conferences: this is a worldwide conference talking about everything having to do with the ketogenic diet. It involves people with cancer research, sports, Glut1 Deficiency, epilepsy.
- European Glut1 Deficiency Organization Conferences

Most interesting issues we have been talking about:
Glut1 Deficiency is characterized by epilepsy, movement disorders, and cognitive/behavioral disturbances. We also have variants to this such as with Glut1 Deficiency-like syndrome and stroke-like symptoms as a new presentation and we are trying to make sense of how this fits into this story of Glut1 Deficiency. One of the most important things we have learned over the past 5-6 years is that as pediatric neurologists, our children grow into adults and suddenly the adults have different clinical presentations than the children or the babies, so it is a condition with many phases and it's time dynamic. For us as physicians and researchers, that means looking for different ways to identify the problem and identify the patients. Something else we have learned as our patients get older is that they don't just have a constant movement disorder, they also have paroxysmal movement episodes - meaning sudden, unexpected events having to do with movement.

In retrospect, we have seen these babies doing funny eye-head movements but we put it down as single events that had nothing to do with Glut1 Deficiency, but now we do understand that it is probably the first clinical presentation and we are now spreading the news among the professionals to look for these head-eye movements and then think of Glut1 Deficiency.

There is a new assay involved where you have the red blood cell and you use some fluorescence to make it shine light and then you can measure that light and see whether the Glut1 transporter is there.

Another interesting finding, published by Dr. Monani in 2017, is that the brain vessels really are affected by Glut1 Deficiency. If you have a mouse with Glut1 Deficiency and you treat the mouse with the vector and the gene, you can then rescue the brain microvasculature and that was an important observation to understand the mechanisms of Glut1 Deficiency and then maybe be on the way to find treatment for that.

A paper we published in 2018 followed 10 patients for 10 years in Germany and the motivation behind this paper was that we are all concerned with the long-term side effects of the ketogenic diet being high in fat, thinking about arteriosclerosis and cardiovascular problems. The good news is that at least in these 10 patients, we saw that before treatment and 10 years on treatment, the cholesterol and triglycerides were perfectly normal. An ultrasound of the arteries supplying the brain didn't find any evidence for arteriosclerosis. It's only a very small study but it is the only study that has systematically been following patients for 10 years and its encouraging. We need more data but it helped us address that question.

10 patients, 10 years – Long term follow-up of cardiovascular risk factors in Glut1 deficiency treated with ketogenic diet therapies: A prospective, multicenter case series

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Upstream SLC2A1 translation initiation causes Glut1 Deficiency
In previous work supported by the German Glut1 Foundation, we found was that in a region where you don't usually look - that is called an untranslated region - we found a mutation and we were able to show that it truncates the Glut1 gene so it can't be read properly and that is another disease-causing mechanism. Therefore, you may have classical mutations in the Glut1 gene, you may have compound mutations where you have two genes being involved from father and mother, but you might also have problems in regions that you may not have looked at yet and this is another part of important research to understand where the Glut1 Deficiency comes from.

Glut1 as a protein has certain functional domains, areas where we now understand how the protein, how the transporter is working. You have the transporter weaving its way through the membrane and you have glucose that has to enter the cell. In order to do so, you need an anchor (an intracellular loop in the middle of the protein so that the protein will stay in the membrane), an amino terminus (determines the half-life and the trafficking), an antenna (regulates the protein), energy binding sites (very important for opening and closing the sugar channel), and the carboxyl terminus (substrate recognition site, that's how the glucose transporter knows that glucose is the substrate to transport). If you have a mutation there, the transporter is blind to glucose. It will be there but it will not understand that glucose is the substrate it should transport. With individual mutations of patients, we can then find explanations as to why in this individual child we have Glut1 Deficiency and this is always the first step to finding treatment.

In an important paper published in 2014, they crystalized the structure of the human glucose transporter Glut1 and showed the 3-dimensional structure of it. What they also did was put in mutations of Glut1 Deficiency patients to see how in this 3D model the glucose transporter is impaired and what they found is that there is a certain amino acid (N34). It affects the pore where the glucose runs through the protein. If you don't have that amino acid and you replace it by another, the glucose channel will be blocked. The transporter will be there but it will not be able to transport the glucose. All these mechanisms are important to understand because they eventually lead to therapy.

There are about 400 patients now that have been described but there are more out there. It is important that parents go into the registry that Dr. Pascual has set up and give us information on your children. Most of the patients are identified by genetic testing now, not the lumbar puncture. The brain MRI does show white matter abnormalities and that is something we have to look into because we haven't really anticipated that. In regards to the treatment, antiepileptic drugs (AEDs) are usually ineffective, a ketogenic diet, including a Modified Atkins diet, is effective in 2 of 3 patients.

The most important message is that the earlier you do the diagnosis and the earlier you start treatment, the better the outcome. It's not just which mutation you have (whether it's a missense or a nonsense or a deletion) that makes a difference but it is also the timing of when you get the diagnosis and when you get the proper treatment.

Consensus

After 28 years, it's time to bring all the experts together and give you and the scientific community an expert opinion of what we all think are the issues that we agree on in Glut1 Deficiency and where are the controversies, what are the problems we anticipate.
It is the combinatorial work of an international expert panel and what we are talking about is the clinical presentations (epilepsy, movement disorder variants), how to do the diagnosis, how valid is lumbar puncture and genetics, which assay should you do, the treatments, ketogenic diets, the ketoesters and oral ketones, anticonvulsant medication, the research and future perspectives.

Some of the questions being addressed:

How do you really confirm the diagnosis of Glut1 Deficiency?
If you have a patient that has the classical symptoms of Glut1 Deficiency (abnormal lumbar puncture, hypoglycorrhachia, low CSF lactate, and a mutation in the SLC2A1 gene) it is Glut1 Deficiency and no one will argue that. However, what happens if you have patient who does not fulfill all of these criteria? The clinical presentation can be shown in isolation and in combination and depending on the presentation, it could be suggestive of Glut1 Deficiency.

What is the proper diagnostic workup?
Is gene sequencing necessary for a diagnostic workup?

All agreed that genetic analysis is necessary and most would also do cranial MRI imaging and an EEG.

What is different between the ketogenic diet for Glut1 Deficiency and in intractable epilepsy? This is an important question because there are guidelines for how to do the ketogenic diet in epilepsy and for Glut1 Deficiency there are some specific differences. The treatment is optional in children with epilepsy but it is the treatment of choice for children with Glut1 Deficiency. With epilepsy, the ketogenic diet can be discontinued after 2 years but the same is not advised for Glut1 Deficiency. We all agree it should be started as early as possible and the monitoring of side effects is much more pronounced in the long run of Glut1 Deficiency. The modified Atkins diet (MAD) is an alternative in adolescents and adults or if you have incompliant patients. There is debate on whether or not the higher the ketosis, the more beneficial the ketogenic diet.

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Natural History and New Challenges Uncovered

Failure of the Ketogenic Diet

In all big scientific papers of Glut1 Deficiency, it is generally stated that some patients don't respond to the ketogenic diet. But nothing is said of those patients. Roughly 10-20% of the patients do not respond very well to the diet but nobody focused on those non-responders. It is a difficult issue but it is important to address.

We checked all of our patients and did a retrospective review together with a laboratory center and the only thing that was important for inclusion was an appropriate diagnosis. What we found was that 10% of our patients were not responding to the ketogenic diet. They all happened to be females, they all had an early onset of some generalized seizure phenotype, but compared to other Glut1 Deficiency patients, a late onset disorder. There was a delay in diagnosis of these patients. They all had similar looking EEGs: series of generalized spikes and wave discharges predominant over the frontal cortex and they all had some multifocal epileptic discharges.

Why didn't these children respond?
Two patients did not tolerate the diet so they were unable to continue after initiation. One patient did tolerate the diet but was unable to attain ketosis for unknown reasons. Four patients were perfectly well in ketosis but completely unresponsive to the diet. In those four, we found that their EEG did not improve, it either became worse or remained unchanged. This contrasts what normally happens in Glut1 Deficiency patients that you put on the diet, their EEGs will improve.

Summary:
The ketogenic diet can fail in Glut1 Deficiency. Failure is caused by intolerance, failure to achieve ketosis or inefficacy (no effect despite adequate ketosis). Advanced age at seizure onset and female sex might be potential risk factors for worsened response to the diet. This is disappointing that there are children that do not respond to the diet, and it is necessary to dive into this topic. We need more clinical data on diet failure and we need to try and understand why this very logical and rational approach doesn't work in some of these children.

Lactate as a new opportunity for treatment

Glucose is the major fuel of the brain. It has to be transformed into pyruvate. Pyruvate is the main source of energy driving the citric acid cycle in the mitochondria. Fatty acids or ketone bodies derived from fatty acids can enter the energy factory and use another transport system and that's why the ketogenic diet works.

Lactate is derived from glucose and low lactate is a marker in the CSF of Glut1 Deficiency. Patients with glucose transporter disorder have low CSF glucose and also have low CSF lactate. One could reason that if you don't have a source for lactate, your lactate will be low. But it might also be the case that lactate is used by the brain as an alternative energy source. There is a scientific basis for this - there is a lot of literature about lactate as an energy source for the brain, but it is important to realize that clinicians have considered lactate to be a waste product because lactate is high in circumstances of cardiac failure and sepsis. However, lactate can also be beneficial because lactate can be an energy source for the brain. In fact, lactate accounts for roughly 10% of the energy requirements of the brain under normal circumstances. The higher the lactate in the blood, the higher the lactate uptake and use in the brain. If you do high intensity exercise, lactate levels increase in healthy subjects as well as in other patients to 10-50 mmol/L in blood and you can measure that.

Can we use lactate infusion in the clinic?

A literature review was done about all studies known in the medical literature on the therapeutic administration of lactate in humans. What was found on therapeutic lactate infusion in humans:

- 51 articles were found in clinical practice, not in an experimental setting, all on adults.
- In general, they showed that lactate can safely be given and showed that the brain indeed uses lactate as an energy source, and studied cardiac output failure, hyperlactatemia, and traumatic brain injury.
- If adult patients with cardiac failure are given lactate, it increases their cardiac activity.
- It shows that if you give lactate to patients with hyperlactatemia, the brain does better.
- It shows less signs and symptoms of shortness of glucose and in traumatic brain injury, it saves the patient from high intracranial pressure and seems to lead to better outcome.

From this information, we can conclude that lactate is an alternative fuel and that lactate infusions appear safe. The question becomes would patients with Glut1 Deficiency benefit from lactate infusions?

A study proposal to study this topic is underway. We should choose patients unresponsive to the ketogenic diet because they are at a higher need for a newer therapy. The idea is to simply infuse lactate as has been done in all the other trials of adults and in this case under continuous EEG monitoring. The idea is to run a pilot study to see if it would work, improve the EEG, and improve the seizure frequency. The idea is that it would work immediately, similar to antiepileptic drugs in an emergency state.

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Update on METAglut1 blood test development for the timely diagnosis of Glut1 Deficiency Syndrome

The blood test we have developed is to help in the early diagnosis of Glut1 Deficiency patients. We are a diagnostic company based in Paris, France. METAglut1 is the name of this specialty test. We are a team of 15 people made of, biologists, mathematicians, experts in software developments, working together to develop innovative solutions for early diagnosis of disorders and to provide tools for precise prognosis or monitoring of patients.

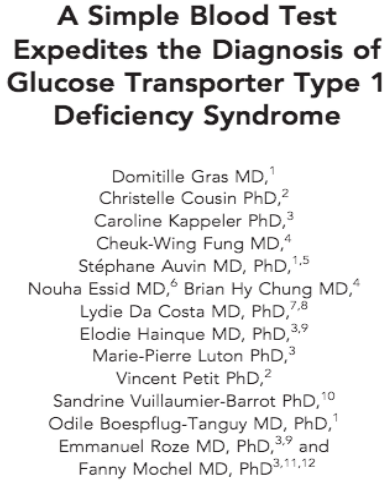
What is of interest to us as a diagnostic company is to understand the way cells are feeding their energetic needs. You all know of glucose but there are other nutrients that can fuel cell metabolism and cell energetics.

Unique ligands of nutrient transporters provide a powerful means to characterize cell energy supply

At the very basis of our technologies is a series of reagents called RBDs, which were derived from viruses and which are able to specifically target nutrient transporters. By using these RBD which bind specifically to these nutrient transporters, we can quantify the cell expression of these key players of cell metabolism and try to decipher cell status. We assess whether a cell is consuming nutrients at a proper level or whether a cell is over consuming certain nutrients (that can be the case for cancer cells) or whether it is suffering from starvation as is the case with Glut1 Deficiency.

METAglut1 is an in vitro diagnostic (IVD) device used to help in the diagnosis of Glut1 Deficiency

The first disorder for which we have developed a test is Glut1 Deficiency. We have taken advantage of the high level of expression of Glut1 on red blood cells and by using the RBD specifically recognizing Glut1 we can quantify its expression on these cells. By applying this approach to a first series of patients we were able to demonstrate the normal expression of Glut1 to be in a range of 80-120 with the mean at 100. A decrease in Glut1 expression is very specific to the Glut1 Deficiency. It is therefore a robust biomarker to help looking for Glut1 Deficiency patients.


A Simple Blood Test Expedites the Diagnosis of Glucose Transporter Type 1 Deficiency Syndrome

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As soon as there is a decrease of more than 20-25% in the Glut1 protein expression on the red blood cells, the test is labeled positive. Specificity and sensitivity are very good but for sure this has to be confirmed and we are currently running a large validation study in France.

We have made important efforts to make the test available to other laboratories and then comparing the data obtained by different users of the test. The precision is very good and the reproducibility was nearly perfect with less than 2% difference in the quantification of Glut1. The results are generated by an algorithm that automatically computes the output of the cytometer used to run the test.

What is going on right now

After the pilot feasibility study on 30 patients, we are now working with more than 35 hospitals across France included in the validation study. The promise of the test is to help physicians test for the disease as soon as possible i.e. as soon as they suspect Glut1 Deficiency. Upon presentation of clinical symptoms, patients are included in the study. METAglut1 is performed blindly on all these patients.

Inclusion

We started the study in September 2018 and there is mainly a prospective cohort (new suspicions of Glut1 Deficiency) of more than 120 patients to date as well as a retrospective cohort (already diagnosed Glut1 Deficiency patients) of almost 45 participants. In terms of age, most patients are quite young, below 12. We try to encourage including patients as soon as possible because the promise is also to help shift the mean age of diagnosis towards babies and newborns to initiate the ketogenic diet as soon as possible. One third of patients present with classic phenotypes and two thirds have other phenotypes.

The test was approved to for use in Europe (CE mark) and contacts have been initiated to get the FDA approval for use in the USA. The next step will be to modify the test to be able to implement it for newborn screenings. We know that from a technical point of view it is feasible, but there is more work required to switch from a test designed for symptomatic patients to a test done on a drop of blood from a newborn baby.

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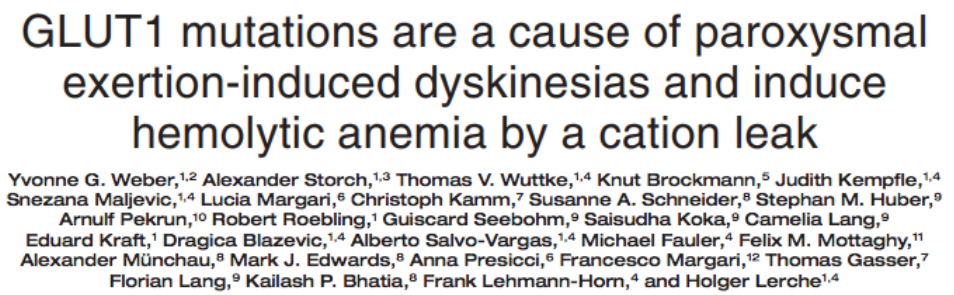
Movement Disorders in Glut1 Deficiency

As movement disorders neurologists, we recognize important features of movement disorders in Glut1 Deficiency to help us make the diagnosis. There is a spectrum of symptoms that can be found in Glut1 Deficiency - epilepsy, movement disorders, learning and attention difficulties, and episodic neurological symptoms are some of the key features of the disease that help us put the puzzle pieces together when we are trying to make a diagnosis in a patient and recognize that that patient has Glut1 Deficiency.

Historical Background
The original description of this condition was in 1991. In the first decade or so of the disease, the feature that stood out most to people was the refractory infantile seizures. In 2008, movement disorders become a lot more prominent in our thinking about Glut1 Deficiency and that is because there was a paper that was published discovering that the Glut1 gene was the gene that caused a movement disorder condition that is called paroxysmal exertion-induced dyskinesia. If we break that down:
Paroxysmal = an episode
Exertion-induced = exercise induced
Dyskinesia = involuntary movements


GLUT1 mutations are a cause of paroxysmal exertion-induced dyskinesias and induce hemolytic anemia by a cation leak

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Movement disorders are a major component of Glut1 Deficiency and sometimes they can be the predominant symptom or even the only symptom. The movement disorders have characteristic features that can provide the key to diagnosis. They also evolve over time so the problems that may be there in infancy or childhood may be quite different from the ones that happen later. The response to treatment is variable. Some of the types of movement problems respond well whereas others respond less well.

It is important to think of there being both persistent and episodic symptoms in movement disorders in Glut1 Deficiency. This is one of the hallmarks of Glut1 Deficiency.

Persistent movement disorders

***Spasticity*:** leg stiffness, toe walking, overactive reflexes in the legs. This is a fairly consistent feature, it doesn't fluctuate much with time.

***Dystonia*:** can look like spasticity because it can also make people stiff but dystonia is much more apparent when someone initiates a voluntary movement. What dystonia does is that the brain sends signals for involuntary postures and movements that go along together with the voluntary movement that the person was trying to make. It has a pattern of being better at rest and worse when the person is moving.

***Ataxia*:** balance difficulties, unsteadiness, and incoordination. In Glut1 Deficiency, ataxia has a very characteristic feature that is not seen in too many other disorders and that is how much it fluctuates. In many patients with Glut1 Deficiency, often times parents will describe some days as being better than others or some parts of the day being better than others.

Episodic (paroxysmal) movement disorders

This can take many forms. One type of episode we see often times is people losing strength almost having what looks like a temporary stroke. Patients that present with weakness on side of the body or both (it comes and goes).

The ataxia can fluctuate a lot. In some patients who have very little at baseline, it can look like they have something called episodic ataxia where they just have these episodes where they lose their balance and in between look quite steady.

Paroxysmal exertional exercise-induced dyskinesia (PED): these are episode that occur after classically prolonged periods of exercise. Exercise is not the only trigger, sometimes it can be fasting, illness, or stress.

Episodic eye-head movements in infancy

It is important to note that there are also episodic symptoms that are not related to movement that happen as well (i.e. crying, migraines) and asking about that in the patient history is also very informative.

A study was conducted with Dr. Roser Pons to try and understand these eye movements, and we reviewed records from 101 patients in the Columbia University cohort and found 10 video examples of patients who had episodes like this. There were 18 patients that had quite a good description of the movements such as what triggered them and what the movements looked like. What became apparent is that this is something that begin in early infancy: in 85% of the patients the episodes started within the first 6 months of age. The youngest started at 1 month. It's a phenomenon of infancy and early childhood because in all but one of the cases where we had that information available, the episodes had resolved by 6 years of age.

Opsoclonus used to be the main term that had been applied to these movements but the eye movements in Glut1 Deficiency have quite different features from opsoclonus:

Eye-head movement characteristics:
Eyes and head move together in multiple directions
Movements are quick, not slow tracking movements
Occur at a frequency of about 1-2 times per second and in between the movements there is a brief period where the eyes are still

Opsoclonus characteristics:
The head is very still and it is just the eyes that are moving
The eye movements are quite striking because they are flickers of movement going back and forth in multiple directions with no break in between

Because of the involvement of the head and the eyes, we decided that the best characterization of these movements is gaze saccades.

Gaze: looking steadily or intently at something

Saccade: a rapid movement of the eyes between one visual target and another visual target

In a gaze saccade, there is a shift in the visual attention to a new target. These movements in themselves are not abnormal, we all make these movements all the time. Gaze saccades are normal movements in the appropriate context but what's different in Glut1 Deficiency is that it happens in discrete episodes that last a few minutes and not followed by visual targets.

Why may gaze shifts occur inappropriately in patients who have Glut1 Deficiency?
The neuronal circuit for eye movements is well understood, better than other movements such as reaching for something. There is this one population of cells in the region of substantia nigra pars reticulate: that population of cells is working extremely hard all of the time because its role is to be a break and tell the eyes not to move. These are very rapidly firing cells that are active continuously to tell the eyes to hold still.

We can start to speculate that maybe this is something that would be vulnerable to not working normally if the brain does not have enough glucose. In thinking about why we see this phenomenon in babies and not in older people, if we think about the normal development of an infant, these movements are occurring at a time in development when babies are developing visual attention. This is probably a circuit that is under active development at that age and requires a lot of resources at that time of life and perhaps later on it requires less.

Sometimes these eye movement episodes are the first symptom of Glut1 Deficiency. In many cases, babies might otherwise look very healthy, like a normally developing baby but this is a huge opportunity to make the diagnosis in early infancy if people do have an awareness of what the significance of that episode is.

In a series that we analyzed, 16 of the patients had both eye movements and seizures. In 10 of the patients, the eye movements came before the seizures, so there is a period of anywhere between 1 month and 12-18 months between the eye movement episodes starting and the seizures starting. If the eye movement is recognized, there is the opportunity to make a much earlier diagnosis.

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Seizures in Glut1 Deficiency

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity
3 Groups of Seizure:

- Focal onset
- Generalized onset
- Unknown onset

Generalized onset seizure: spread of discharge everywhere in the brain
Focal onset seizure: from one area of the brain and the symptoms are related to where the seizure is discharged. Focal and generalized seizures have motor and non-motor onset.
Reflex myoclonic seizures: induced by touch and looks like a jerk. It can also be induced by a surprise.

Myoclonic seizures are always something special for a child neurologist because that jerk-like movement is difficult to say if that it is a seizure. The only way to be sure that it is a seizure is to record the patient and make sure that at the time the patient has a jerk, there is a spike in the EEG. If you have correlation between the motor event and the EEG abnormality then you have an epileptic event, namely a myoclonic seizure. Another type of seizure is myoclonic absences which are characterized by repetitive jerks with generalized spikes and waves.

How we go further from some symptoms to an epilepsy diagnosis:
The child neurologist has to collect at what age the seizure starts, if there is one of several seizure types, what is the clinical examination? A big part of the job is to make sure you understand the clinical history of the patient. If you are able to record the event, you can properly identify what type of seizure it is.

Childhood absence epilepsy (CAE):
An epilepsy that is representing 10% of the epilepsy syndrome in pediatric ages. 1 in 10 patients with epilepsy during childhood have childhood absence epilepsy. They occur around 4-12 years of age and they should have only one type of seizure, absence seizure. They can have up to 100 seizures a day, and one third of them have attention deficits. When you treat them, 4 or 5 are good responders to the first line of treatment. Early onset absence seizures look similar to childhood absence epilepsy but if the patient has any other type of seizure, it is not CAE and Glut1 Deficiency should be the first diagnosis you think of.

Early absence seizures in childhood: absence-like but not true childhood absence epilepsy. In a recent paper, they look at 43 patients that do not have typical childhood absence epilepsy and what they accept is:

- Early onset (before the age of 4)
- Intellectual disability (most of the patients with CAE have normal development)
- Other type of seizures
- Movement disorders in addition to absence seizures

Out of the 43 patients, they found 2 (meaning about 5%) with a pathogenic variant in the SLC2A1 gene. If you associated intellectual disability with earlier onset plus movement disorder, the chance to have Glut1 Deficiency is higher. Even if it appears to be childhood absence epilepsy with only early-onset seizures, a physician should think of Glut1 Deficiency, and if you have any other symptoms, the chance, unfortunately increases.

What type of seizure can be seen in Glut1 Deficiency?

Any type of seizure can be seen in Glut1 Deficiency, even focal onset seizures can be seen in patients. The diagnosis should not be done based on the seizure type, as any seizure type could be seen in Glut1 Deficiency patients. However, if you identify several seizure types plus a medical history that is consistent with Glut1 Deficiency, you should think of Glut1 Deficiency as a diagnosis. This is also true for some epilepsy syndromes where Glut1 Deficiency is frequent like childhood absence epilepsy syndrome or CAE-like syndromes. In any epilepsy with myoclonic seizures, the patient deserves an investigation for Glut1 Deficiency because it is a treatable condition and you have to do the diagnosis as soon as possible.

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Cognitive Considerations in Glut1 Deficiency

- What do we know about cognition in Glut1 Deficiency?
- What skills are preferentially affected?
- What do we think those cognitive skills might be related to in terms of other biomarkers we might have?
- What do we know happens over time when we try to follow the cognitive skills?
- What do we know about behavior and about adaptive skills?
- What can we do to try to help improve the quality of life of the individuals affected?

What we know about Glut1 Deficiency is that it's a complex phenotype that involves a lot of different aspects to it and certainly the cognitive delay is something that was noted early on and that we know is involved in the individuals that are affected. What makes it complicated to study them perhaps in part is we know they are quite variable, and we also know that the seizure and motor disorders that go along with it all can impact how we think about cognition and how cognition develops.

We know the presentation is very variable. We know that the promoter for the gene is mutated which affects the actual transcription and the product that is being made. We know that somehow that affects the structure of the brain such that it affects the function of the brain, and we understand that the function of the brain is not the same as what we see in most individuals. We know from some of the PET studies that have been done that there are specific areas of the brain that are more likely to have decreased energy and so maybe those areas of the brain are more likely to sub serve some of the cognitive skills that we know are involved. However, that step from function of the brain to cognition is an area where we know very little. How does it translate from one area to the other? We are still at the level of trying to understand what is the cognition that is involved. Are there areas that are specific to the disorder that might be more likely involved than others?

There is a lot that we don't know, but the important thing that we do know is that at every step, different environmental factors can have a huge impact and those environmental factors can affect long term outcome. It is important to remember that there is so much we can do to improve outcome because there are things that we can do to help them along the way.

In general, we work with tests that have been given to thousands and thousands of people and we look to see how individuals perform relative to other people of their age. What we see when look at a sample of children who have been diagnosed with Glut1 Deficiency is that overall intellectual function is shifted downward from the general population. But what is most striking about this pattern is that it is quite variable, there is quite a range so it is not like

everybody is the same in any sense of the word. There was a recent paper that came out this year and they looked at a group of 24 patients using a slightly different measure but they found the same thing. A selection of their population was in the normal intellectual function range and then there was a selection of their population at different levels. Overall, relative the general population, the group was shifted downward but there are still individuals with the diagnosis who are performing within the normal limits, they were performing as one would expect for a child their age.

Overall cognitive profiles in patients with GLUT1 Deficiency Syndrome

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They also found that there was a strong correlation between their IQ scores and their overall CSF blood glucose uptake ratio.

Are there specific cognitive skills that are more likely involved in children with Glut1 Deficiency?

It is complicated to figure this out because there is a wide range in terms of overall function and it is also complicated because we work with some children who have been on the diet a long time, who started early, others who started later. We have a lot of variables including age. Some people come in who are 3 and others come in who are 40. To try to aggregate it all and come up with some general statements is complicated, but what we have done is come up with extraordinarily simple measures to try and get to the core of what we think is going on.

Language ability

In children with Glut1 Deficiency you may see differences in terms of their speech. This is something that has a big impact on their quality of life because often times people have a hard time understanding what they are saying. Often times the parents can understand what their child is saying, but others may find it more difficult to comprehend. That makes understanding, working with, and trying to evaluate a child's language skills a little bit problematic because if we can't be 100% sure of what their actual expressive speech is, it's difficult to understand what they might know.

One thing we have done is extraordinarily simple tests to look at what we call *receptive vocabulary* and *expressive vocabulary*.

- **Receptive vocabulary:** you show them 4 different pictures, you give them a word, and then ask them to point to the correct picture for the word (i.e point to sleeping). This is an extremely effective measure that gives us a sense of vocabulary acquisition and often times is strongly associated with overall intellectual function, so it gives you a good sense of vocabulary acquisition.

- **Expressive vocabulary:** the ability to look at a picture and name words, which increases over time

These are both very simple tests, but they're both tests where we don't have to rely on too much motor responding, and we don't have to rely on understanding articulation that well in order to assess.

There are also tests that are good at looking at people across the age span. What we found was that overall the performance as a group is a little bit lower than one would expect from the normal population. But perhaps what is a little bit more striking is that the level of receptive (the understanding of words, i.e. I tell you a word and you tell me which picture represents it) is stronger than the ability for the child to name the picture. That tells us that there is a real difference in cognitive skills. This is a way of confirming that when parents say, "I think he understands more than he says", that it is actually the case.

Cognitive skills

- **Visual matrices:** you see some pictures and try to determine how things fit together appropriately.
- **Visual matching:** you see a picture and you have 4 choices in which you have to determine which choice matches the picture you see.
- **Drawing:** simple task of copying a drawing such as a circle or square.
- **Peg placing:** ability of putting small pegs into a hole rapidly.

These are all tasks that have been normed on the regular population so we know what we would expect for most children of their age range. Generally speaking, what we see is that relative to the population, Glut1 Deficiency children score a little bit lower, but we are definitely seeing particular difficulties with things like peg placing. This is not surprising given what we know about motor disorders. The other thing that we see that is lower is drawing, which is also a fine motor task so it could be that they are having a hard time replicating pictures because their fine motor skills are problematic. Visual matching is also a little bit lower than expected and that is interesting because it suggests that maybe visual attention to detail is not quite as strong as we want it to be. Visual matrices is an area that is actually one of the strong points. This requires looking at pictures and reasoning about to how they are related to one another and that seems to be an area that is stronger.

An example of the drawing task could be to show the child an asterisk and have them copy it. Often times what happens is that they draw the three lines that are not in any way bisecting one another. It is clear that they are attuned to the actual details in the picture but they're somehow not integrating those together. This becomes a problem of visual motor integration: taking visual information, putting it into your head and integrating it. Developmentally, very small children often make the same sort of response but to see it in older individuals is quite striking, and we saw it repeatedly in our Glut1 children. They are having difficulty integrating the visual information they are looking at.

Kaufman Assessment Battery for Children
We chose to use a particular type of test for the evaluation for the reasoning being that it takes into account different processing styles and it is more appropriate for lower functioning children and doesn't rely heavily on language. It is based on the idea that there are different ways of processing information:

- **Sequential processing:** suggests that the way your mind works is that it deals with information coming in step by step by step. (i.e: I say some numbers, you say them back to me or I say some words, you point to the words in the order I said them)

- **Simultaneous processing:** you take the information in at once and put it together. (i.e: form the whole from the pieces or here's a picture, it's not entirely all there but focus on it and tell me what it's a picture of)

What we found is that there is a real difference in terms of processing style amongst these individuals. We found, again, that overall, they scored lower than the general population and it was quite variable amongst our group. Overall when we looked at group aggregate data, we saw that individuals performed more poorly on simultaneous processing and have a certain strength in sequential processing.

Amongst the Glut1 Deficiency individuals that participated, the more holistic and spatial integration strategies were particularly affected, so there is something specific about those findings that maybe is affecting how they process things. We do not know for certain but we can conjecture that what we know about the brain in Glut1 Deficiency is that there is a diminished energy use within the thalamus and the thalamus is traditionally known as an area that integrates information, so it is possible that this is somehow contributing. We can use this in terms of what we call scaffolding techniques in education. What we recommend is teaching them step by step by step and helping them learn it as they go along.

When we look to see how our cognitive skills relate to other skills, we see that they are very strongly associated with the CNS score which is a score of neurological function that was developed at Columbia. There was a hypothesis that maybe there was a difference between gender but in terms of intellectual performance, we haven't seen anything to suggest that there is any difference between males and females.

What happens to cognitive skills over time?
We know with some cognitive disorders that there is a loss of skill over time and so we are always vigilant to determine is this is something we need to be concerned about. The research is fairly conclusive that we don't have to be worried about loss of skill over time for those individuals who are on the ketogenic diet.

We looked at a longitudinal study of a group of 32 children and there is no evidence of cognitive decline over time. They show that they are developing at the same rate as other children their age, but it is just that they started off at a lower level. When we look at the

vocabulary reception test, you can see that they are learning more words over time appropriately. They are a little bit lower than their peers but they are developing their vocabulary knowledge at the same rate as their peers.

De Giorgis has also looked at some questions of longitudinal data. De Giorgis et al., 2019 showed that there were improvements in cognitive function after introduction of the ketogenic diet. What they showed was that the biggest gains were made on the verbal measures, so it may be that with introduction of the ketogenic diet, it is affecting particular domains. You can see from the data that those who had an earlier start to the ketogenic diet had better cognitive performance.

Behavior

Adaptive Behavior: behaviors the individual normally engages in on a daily basis and these are reported by the parents

- **Communication:** how well they speak to other people
- **Daily living skills:** things like making the bed or brushing their teeth
- **Socialization:** how likely are they to interact with others

In general, parents reported a shift downward in terms of adaptive skills, but there were many parents that reported that the skills are within the normal range, so it is a very variable phenotype. What we see is that these scores are very strongly correlated with our intellectual function scores which stands to reason because when you actually diagnose intellectual disability, you have to have the combination of low performance on an intellectual tasks and low performance on adaptive skills. These two different components are necessary for a diagnosis.

If we break down the adaptive skills into the three domains: communication, daily living skills, and socialization, we see a remarkable finding of very strong social skills. It stands out as an area that consistently is stronger than one would expect given other levels of performance.

As children mature they may be at an increased risk for adjustment difficulties associated with living with a disability and therapy may be beneficial for navigating that.

Summary

- Overall cognitive function was shifted downward from the general population.
- Receptive language skills are stronger than expressive skills.
- Visual attention to details is weak.
- Fine motor skills are weak (i.e peg placing task).
- A stepwise approach to teaching material is recommended.
- There seems a bias in cognitive processing style but you can use that to your advantage.
- Developmental gains are seen over time.
- There is no evidence of loss of skill over time for those who are on the ketogenic diet.
- Social skills are strengths.

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Other Symptoms in Glut1 Deficiency

Patient Registry
UT Southwestern has funded and created this registry in conjunction with a generous gift from the Glut1 Deficiency Foundation to collect data about Glut1 Deficiency. The registry is a patient-driven enterprise. It's a worldwide website, and we have a few hundred patients who have registered. It's a pretty significant undertaking to sit down and compile all of the different kinds of information that we are collecting. We want to know everything and anything because it allows us to detect patterns of things that happen or may not happen in ways that any of us individually cannot identify because we don't have the capacity to see hundreds of patients at any given moment. You provide us with the data, and we collect and analyze it.

The problem we have right now is that a number of people have registered but have not completed the entry. The registry is a pretty hefty mathematical project. There is predictive mathematics that go into anticipating, based on who you are, how you are going to do in the future based on a number of variables that you will provide us.

The registry is a moving target. 18% of the patients in the registry are older than 18 years and 5% are older than 30 years and now have 10 times the number of people we knew about 3 years ago. The more data we acquire, the more aware we are in terms of our understanding of the disease.

Less well-known associations:

- Pigment dispersion in retina- these are not obvious eye problems in a normal eye exam; you have to specifically look for this sort of thing.
- Chronic thrombocytopenia from birth
- Persistent hematuria
- Celiac disease
- Congenital brain malformation

Overcoming the limitations of DNA
DNA is an instruction manual, it's not an agent that does something. It tells the cell how to do something but once you have the instruction and do that thing, a whole array of biological events happen to take over, many of which are very independent from the original DNA instruction.

Of 250 patients seen in Rare Brain Disorders Clinic last year with unusual brain problems, (Glut1 Deficiency as well as many other things) only 7% had a genetic cause for the problem. That doesn't mean the majority didn't have anything wrong with their DNA, there may well be

something wrong, but there are too many changes in the DNA to assign the blame to one particular gene. It could be that there are many low-grade changes all of which combine to bring about the problem.

Gene → Function → Health
Gene loss→ Perturbation →Disease
Gene loss →Gain → Enhancement

If you lose a gene, you may actually gain something because the gene is suppressing some function of the organism. If you lose it, you gain some of the function. That may be bad in many cases or it may good in some cases. Therefore, you get an increased capacity on that domain.

What we have been doing is suppressing one gene at a time (of 23,000 genes) in the mouse and determining how the brain responds to that. Is there anything that changes if you knockout one gene or a few genes at a time? We are doing this not only find disease causing genes, but also to determine how many of these gene losses lead to a higher performance than normal. The idea is that you can potentially develop a drug that will do what we did in the laboratory. Most of the drugs actually block gene function. The hope is that if you knock out a gene that gives you a high performance when you knock it out, then maybe you will be able to compensate for the dysfunction.

We have done about 25,000 of these mice by now, individually studied. We have gone through approximately 60% of the entire genome, we go through 3% of the genome per month. We are cautiously optimistic that we should be able to find a handful of these genes that will lead to drug development to be able to suppress them without having to go through any genetic methodology in a human being and hopefully we will achieve the higher capacity than normal, which means someone with a disability can benefit from this regardless of what the cause may be.

Triheptanoin Treatment
The beauty of human brain metabolism is that there is more than one way to get to the same place. What C7 does is to provide a different route to the same endpoint.
There are clinical trials involving Triheptanoin (C7):

1. Define the appropriate dosage
2. Characterize the safety and toxicity
3. Currently trying to see what happens to patients who are on modified atkins diet or no particular diet and the third phase would be looking at people who are on the ketogenic diet and determining whether that is compatible with C7 or not.

Triheptanoin is a triglyceride fat, it has been in the market and the food chain for a very long time. It is a glycerol molecule and has 3 fatty acids attached. They're the same length and have 7 carbons. When it is cleaved in the liver, it produces a 3-carbon unit and two 2-carbon units. There are very few sources of 3 carbon units in human nature. This will make a large quantity of fat and the two carbon units are made by metabolism of other ketones.

It is fueling pre-metabolism through two different mechanisms, so if one is not working very well, it has another source, and this metabolism has been characterized extensively. A few years ago, we did a study where we had a number of children come in and we ran the usual EEG and they all had a reduction in seizure rate with the C7 treatment.

Neuropsychological performance after Triheptanoin

It is also well known that you may treat some kinds of epilepsy, especially absence epilepsy with drugs and you may normalize the EEG, you may indeed treat the epilepsy. Absence epilepsy you can treat with any one of 3 drugs. However, having treated hundreds of children with one of these 3 drugs, and their epilepsy having gone away, and the EEG looking good, the cognitive profile did not get better. They were able to decrease absence seizures but not cognitive improvement, so you can treat the phenomenon that you see, but there are consequences of an underlying problem. What we are doing is making cognitive profile a very important part of the trial, in addition to everything else mentioned, so that we can improve that.

In looking at the EEG, it is important to do a seizure count, look at the phenomena that it shows such as spikes and waves but there are many other features of the EEG that are quite important for things like cognition. There is no doubt that the presence or absence of a seizure is crucial but there are also many other components to the EEG signal that tell you other things that go with the cognitive profile of a person.

A lot of that is underlined by the interplay between excitatory and inhibitory neuron. They have to be communicating very frequently and that communication has to be very precise. The inhibitory neurons produce something that we call gamma oscillations which are very high frequency discharges (40 times per second). In a recent lab publication, high frequency oscillations and inhibition were decreased in energy metabolism disorder pyruvate dehydrogenase deficiency (another metabolic disorder of glucose downstream Glut1). They don’t have sufficient gamma oscillations which are important to maintain the balance between excitation and inhibition, and they are disruptive in a number of conditions, all of which have to do with learning impairment. This leads us to test if C7 energy fuel would modulate EEG high frequency oscillation in Glut1 Deficiency and we see a significant increase in the oscillatory frequency with Triheptanoin.

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Glut1 Deficiency in Adulthood

*Dr. Cervenka will be sharing more in-depth information about the Adult Experiences in Glut1 Deficiency project in a forthcoming peer-reviewed publication.

Introduction to adult Glut1 Deficiency

Who are adults with Glut1 Deficiency?

- Children who were initially diagnosed in the 1990's and are now adults. Right when Dr. De Vivo started describing Glut1 Deficiency, there were many children that were diagnosed right then and there in the early 1990's.
- Adults that began having mild symptoms as children and are now being identified/diagnosed as adults, even though those symptoms may have been going on for years and years.
- Adult onset patients include those where the symptoms actually weren't there in childhood or they were so mild that no one ever paid any attention and now they're starting to have some new symptoms as adults.

Symptoms can be:

- Mild chronic encephalopathy/cognitive impairment
- Infrequent seizures (seizures may resolve by or in adulthood)
- Varying spasticity, ataxia
- Paroxysmal exercise induced dyskinesia

We are learning that symptoms aren't occurring sequentially. They tend to overlap. Some symptoms are present in some patients but not in other patients and some may appear then disappear at different ages while others may be life-long.

Genetics

We also now have adults whose children have been diagnosed with Glut1 Deficiency who then themselves get tested, find out that they have an associated genetic mutation with or without symptoms. This challenging situation brings up whole new questions of if or how we should treat these adults. We know that the genetic inheritance pattern can be autosomal dominant. We also know that there are de novo mutations being discovered, so we are getting more information about the varied phenotypes of children and adults with Glut1 Deficiency.

Brain Metabolism in Adults with Glut1 Deficieny

As the brain ages, it requires more energy up until about 6 years and then the energy requirements decrease over time and eventually plateau. As a result, we don't know target blood ketone levels and which diets we should recommend for adults, which is why it should be on a case by case basis based on the symptoms, the patient's willingness to follow a ketogenic diet therapy, family, and social support.

Adult Experiences in Glut1 Deficiency Project
This was a first ever large-scale survey of adult patients with Glut1 Deficiency. This is different from the registry in that it focuses solely on the experiences of adults with Glut1 Deficiency. The goal of the project was to identify gaps in knowledge, care, and services provided to be able to start fostering discussions and building resources.

The survey had over 55 questions asking about demographics, symptoms, treatments, therapy services and social and emotional support.

- Over 50 surveys were completed describing the experience individuals with Glut1 Deficiency
- Over half were completed by a care provider

Results
- Half of patients were female and half were male.
- Average age was 28 years old (range was 18-50 years old).
- Age at diagnosis average of 18 years (range 8 weeks-49 years).

Symptoms that were reported by greater than 50%
- Cognitive difficulties
- Ataxia
- Speech difficulties
- Dystonia
- Seizures
- Paroxysmal exertional dyskinesia
- Mobility issues
- Memory difficulties

Triggers
Over 80% of patients or families reported one or more triggers for their episodes. The most common were:
- Emotional stress, excitement, anticipation
- Exertion
- Warm weather
- Hunger
- Fatigue

Hormones

- Two-thirds of men and women combined reported changes in symptoms at the time of puberty
- One-third reported worsening and fewer reported improvement
- Nearly one-third reported new symptoms
- The new symptoms tended to be more of the movement related symptoms, migraines, and mood disturbances rather than new-onset seizures.
- The majority of women reported changes in symptoms associated with their menstrual cycle.

This is something we are very interested in because there is a kind of epilepsy called catamenial epilepsy so we certainly know that seizures can be associated with the menstrual cycle in adults with epilepsy of all types.

We are trying to understand the correlation between seizures and ketone levels and hormones because the ketone levels can fluctuate with the hormones. Over 80% of women of childbearing age reported worsening of symptoms around menses; this included seizures, fatigue, and migraines. Very few reported improvements and some even reported new symptoms that only happened around the time of their menstrual cycle.

Dietary Therapy

- Over 60% of the patients were on the ketogenic diet therapy
- Approximately half of the patients were on the classic ketogenic diet, followed by Modified Atkins, followed by modified keto.
- Very few were on a low glycemic index diet, and 3% were on the medium chain triglyceride oil diet
- One quarter were one a regular diet.
- Other diets included a regular diet with Triheptanoin and a regular diet with corn starch.

One of the statistically significant findings from this survey was that if you looked at patients that had a history of childhood seizure disorders that were on a ketogenic diet and compare them to those patients with a history of childhood seizure disorders, the ones that were on a ketogenic diet had a statistically significant higher chance of being seizure free.

Continuing Care

- Less than half of all respondents are being seen by an adult neurologist
- The majority are being seen at a ketogenic diet center
- Nearly half have discussed coming off the ketogenic diet
- The majority are seen by an adult dietitian or nutritionist and have discussed switching from the classic ketogenic diet to Modified Atkins diet
  (the Modified Atkins diet is going to be better than not being compliant on a classic keto diet)

Regarding whether or not to ever stop ketogenic dietary therapy:

- Very few respondents plan to stop the diet, and nearly half do not plan to stop
- Half are undecided

Seizure outcomes

Patients that are seizure free were younger than the patients who were not seizure free. There was no difference with regards to gender and likelihood of seizure freedom. Nearly half or respondents are on anti-seizure drugs.

Therapy services

- Almost all respondents reported using therapy services at one time
- Fewer than half have therapy now
- Approximately one third have speech and occupational therapy right now
- Nearly all respondents reported that there were physical activities (i.e walking, jogging, running, swimming) that reduced their symptoms.

Educational, social, and emotional support

- The majority attended secondary school
- Over half receive disability benefits
- Half do volunteer work
- Fewer than half work part-time
- One third attend a day program
- Over 1/4 attended vocational school
- Fewer than one quarter work full-time

Daily Living

Adults with Glut1 Deficiency could independently complete one or more of their activities of daily living (household chores, managing finances, driving, cooking)

- The majority live with family
- Over one third handle their own transportation independently
- Over one quarter are dating, engaged, or married
- Several have children, all of those children have also been diagnosed with Glut1 Deficiency

Key findings

- There is a wide spectrum of abilities, clinical symptoms, and responses to treatment in adults with Glut1 Deficiency.
- Emotional stress seems to be the most common trigger.
- Physical over-activity may provoke symptoms but other physical activities may reduce symptoms.
- Patients on ketogenic diet therapy are more likely to be seizure free as adults than those who are on a regular diet.

Opportunities based on these findings

- Improve availability and education of adult neurologists to care for adult patients with Glut1 Deficiency.
- Identify appropriate treatment options, specifically determining the optimal ketogenic diet therapy across the ages and whether or not to stop the diet.
- Improve availability of therapy services for adults.
- Identify training and employment opportunities.

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Treatments Old and New

The treatment of choice is the classic ketogenic diet. We do know that now the Modified Atkins diet is used by many patients. Acetazolamide has been mentioned as one of the drugs.
An observation reported by Dr. De Vivo is that you could also approach it from another angle and not supply a lot fat for ketones but try to keep the glucose levels as high as possible to try to provide the brain with energy. The triheptanoin study is ongoing.

We know of 19 adult patients with babies and they all have Glut1 Deficiency, so there are a lot of open questions about therapy. Maybe the mother being pregnant and being on a ketogenic diet has a protective effect for the unborn baby? Maybe once the baby is born and you know that it has a mutation and has Glut1 Deficiency and you put it on the ketogenic diet, then immediately it may have a very beneficial effect? This is something we need to look into.

Eric Kosoff, MD recently updated the recommendations on the ketogenic diet treatment from international consensus.

Optimal clinical management of children receiving dietary therapies for epilepsy: Updated recommendations of the International Ketogenic Diet Study Group

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What we recommend for Glut1 Deficiency is that babies go on a 3:1 ketogenic diet until the age of 2 years. In school-aged children the classic ketogenic diet is the diet of choice, but then you can see the Modified Atkins and low glycemic index diets kicking in. So far it is not recommended for younger children but things are changing and we are constantly learning new things.

Looking at a study published in 2016 with 92 patients with Glut1 Deficiency, the majority do use the ketogenic diet (in western countries). The Japanese think a little bit differently and they recommend the Modified Atkins diet over the ketogenic diet, and the interesting thing is that seizure control is the same. However, they didn't look at many other important aspects of the disease such as movement disorders, paroxysmal events, development, and speech. Seizures are just one part of the story and still the question is unanswered as to which diet is the best.

Glut1 mistrafficking: a transport gone wrong

If you have Glut1 assembled and have a dileucine motif (2 leucine amino acids), this causes clathrin, a protein, to take the Glut1 transporter from the membrane, where it should be, into vesicles and it will be brought into endosomes and will be disabled and disintegrated. This is an example that if you have these dileucine motifs in your Glut1 transporter, then it reaches the membrane, but then it gets taken away and dissembled. If you could stop that process by having a clathrin-dependent endocytosis blocker, a drug, then you could stop it there and it would leave Glut1 in the membrane. This is just theory, but it's an example of how complex mechanisms can be and if you could stop that process by a drug then maybe this would be an opportunity to improve Glut1 expression on the membrane.

Acetazolamide

In a single report in 2011 on a patient with paroxysmal dyskinesia, they gave him Acetazolamide and everything went away. In the literature, there are 5 papers with 6 patients published so far on Acetazolamide. However, there is a lot of variability among the individuals: the age was all over the place, the mutations they have are all different, the frequency is all over the place, some are on the ketogenic diet and some are not, the dosage of the Acetazolamide across patients is quite different. Additionally, most of them have not been followed so they saw an effect but they never checked whether this effect has endured over time. So as a physician it is difficult to determine if you should be giving Acetazolamide. In practice, I have 6-7 patients on Acetazolamide and it did work on one of them. So yes, it is a treatment opportunity, and we know the drug quite well because it has been around as an anticonvulsant for decades, but still the evidence that it really helps is not as suggestive.

Anticonvulsant therapy in Glut1 Deficiency

Experts have concluded that current data on anticonvulsants therapy in Glut1 Deficiency was rated insufficient to provide any recommendations. We still we have patients with seizures and we still use anticonvulsant drugs, but we don't have a good understanding of which drugs to use first or in what combination.

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Anticonvulsants in Glut1 Deficiency

We have to deal with the question, which one of the available ones are the best and are any drugs of particular problem.

Effects of anticonvulsants:

1. As kidney stones and metabolic acidosis are well reported side effects of carbonic anhydrase inhibitors and a ketogenic diet, it is recommended that urine calcium and creatinine ratio should be measured.
2. If Valproate and ketogenic diet are used together, we have to consider an increased risk of carnitine deficiency.
3. Influence of ketogenic diet on anticonvulsants in children with refractory epilepsy.

It is well known that in Glut1 Deficiency, every medication that inhibits Glut1 function may aggravate neurological functions associated with the Glut1 Deficiency.

Study: observation multicenter case series

- Enrolled 35 Glut1 Deficiency patients at a median age of 2.2 years at the first seizure
- Follow-ups took place every 6-8 months for effect of the antiepileptic treatment
- Seizure free was defined as no reported seizures for at least 2 years.
- In total 49.3% have been seizure free during the follow up.
- In 28 of the 35 patients, the ketogenic diet therapy was applied at a later age and for a considerably longer time in comparison to the other anticonvulsants.
- The cumulative rates for becoming seizure free over time are significantly higher under the ketogenic diet therapy.
- After 30 days of treatment, the rate for being seizure free under the ketogenic diet therapy was 55%. After 100 days of treatment it increased to 71%.
- No significant differences could be found between valproic acid, ethosuximide, and levetiracetam.

Currently there are no recommendations for AED therapy

- Available data confirm that ketogenic dietary therapy achieves best seizure control.
- Ethosuximide and valproic acid seem to be the most effective add-on AEDs.
- Phenobarbital and lamotrigine may potentially reduce the efficacy of KDT.
- On ketogenic diet and valproic acid, patients should be monitored for carnitine levels.

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Gene Therapy

Gene Therapy: From Bench to Bedside
Gene therapy: using viral vectors to deliver relevant genes in the body that are missing

Replacing a defective gene – AAV gene therapy
AAV is a dependoparvovirus meaning it cannot replicate on its own. It depends on another virus called adenovirus for replication. As we know, DNA exists as a double helix, it has two strands but this virus actually is a single stranded DNA virus. This is an engineered virus to deliver the therapeutic gene.

A therapeutic gene is encoded in something known as an expression cassette, which has the gene and is driven by a promoter which will drive the capacity of the gene to be delivered to certain cell types and at certain levels. When we make this virus, the virus is made in a cell so what we do is we take three components, the replication component, the viral capsid, and the transgene in the cell and we make the viruses in the cell. The cells are lysed and what we get out of this is virus particles which are unable to replicate so all it can do is deliver the gene. What happens then is the virus enters the cell and it enters the nucleus and encodes the protein it was originally designed to be encoded.

Target tissue determines the route of administration and dose
Since Glut1 is expressed in two cell types, endothelial cells and astrocytes, we could either deliver it systemically via circulation or we can deliver it directly to the brain using CSF as the route of administration.

Gene therapy has gone through 50 years of milestones and development. There are currently 2 successful products in the clinic:

- LCA2: a rare ocular disease
  There is a significant improvement in the visual cortex activity, meaning AAV has successfully delivered the gene and corrected the defect in these patients. FDA approval was granted in 2017.
- Gene therapy for SMA: a rare neurological disease
  It's a lethal disease where many patients would not make it beyond 2 years of age, but when treated with the drug, they are actually going to school and sitting up. It was approved recently in 2019.

We can target the virus to a relevant cell type—known as capsid engineering. It has already shown that it works wonderfully in mice, and now we need to take it to the clinic by engineering the capsid for humans. We are very close to a point where we can target the right

cell types and we want to express it at the right levels, so that is where the promoter optimization comes into play.

The final hurdle is manufacturing. We know when we have all of the parts of the puzzle in place, we want to make it available in huge quantities to be able to treat a lot of patients and this is a big challenge in gene therapy with the current set up, so we are also working on improving the cellular ability of making these viruses by engineering all the cells and making it a clinical product.

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Ketogenic Diets in Childhood

We are at an interesting time in the ketogenic diet world. Those of us in the ketogenic diet community are seeing an explosion of interest, an explosion of people asking about the diet, both people with epilepsy and without it. We have just come off what many of us in the field would consider one of the biggest years ever other than 1921 when the ketogenic diet was created. So far to date, there have been 3,286 publications on the ketogenic diet and seven randomized control trials. One of the big drawbacks of the ketogenic diet in the epilepsy community for many years was that there were no randomized control trials that proved it worked. We now not only have one but we have had seven, and two of those are in adults.

We just had our first international ketogenic diet meeting outside of either North America or England which showcases how well the diet has become international. We had our second European Glut1 Deficiency conference in June. The American Epilepsy Society for the first time ever last year had an evening symposium devoted to the ketogenic diet. We revised our international ketogenic diet consensus paper: this is a guideline for those centers who are starting the ketogenic diet or maintaining the diet from many dieticians, neurologists, scientists from all around the world.

The research is also expanding. We have seen a lot of research now in the last few years. The number of papers has increased exponentially from around a paper or two per year to a paper or two per week, and this is specific to neurology. The neurology community is very interested in the ketogenic diet.

Hot topics in the keto community

Infants

For many years, using the ketogenic diet in infancy was frowned upon because people would say that infants could not be maintained on the diet, they could not achieve ketosis, they would say it was unsafe to do, and now we have come almost full circle where just this past year we had a consensus paper about how we should best be maintaining the ketogenic diet specifically for infants, with paper after paper showing how well it can be done. One paper out of Austria compared infants to older children and found that infants had better seizure control.

Contribution of the gut microbiome to ketogenic diet mechanism of action

There are lots of theories as to why the ketogenic diet works, not specific necessarily to Glut1 Deficiency, but for epilepsy in general—maybe it's the ketones, the high fat, keeping your blood sugar stable, adenosine, mitochondria— these are all some of the theories that keep popping around. One of the new theories that is extremely intriguing is the concept that maybe when

you are on the ketogenic diet, your gut microbiome changes and maybe those changes may lead to better seizure control through increased GABA, an inhibitory neurotransmitter.

Consensus Paper

Back in 2006 was the first time we decided that perhaps we needed a consensus paper, a guideline that would be helpful for centers that were doing the ketogenic diet. In 2006, we had enough research that we felt it was reasonable to put a document together to back up anecdotal claims (or refute some). The revision was published last summer in Epilepsia Open which is an open access journal (Kossoff et al., 2018).

- No scientific evidence for calorie or fluid restriction
- There is a lot of evidence about all 4 of the ketogenic diets
- All 4 diets are equally valid although we still recommend the ketogenic diet for patients younger than 2 years and MAD for patients over 12 years of age.
- We are seeing less and less centers fasting now at the start of the ketogenic diet (about 28%)

More information specific to Glut1 Deficiency

- 80% of patients have a greater than 90% seizure reduction on the ketogenic diet or Modified Atkins diet.
- Highly effective for cognition and movement disorders
- Ketogenic diet should be used as soon as possible
- Use the classic ketogenic diet in infants and preschool children if possible
- Maintain it as long as possible
- In Glut1 Deficiency, the ketogenic diet is recommended to be maintained longer into puberty and maybe even beyond that

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Ketogenic Diets in Adulthood

There are 5 ketogenic diet therapies that are used for adults with seizure disorders, including Glut1 Deficiency Syndrome. For a diet to be "ketogenic" means that a person following the diet is breaking down fat as fuel, thus producing ketone bodies:

- Classic ketogenic diet
- Modified ketogenic diet
- Modified Atkins diet
- Medium chain triglyceride diet
- Low glycemic index

There have been 2 adult randomized controlled trials, both looking at the effectiveness of the Modified Atkins diet to control seizures. In one study, there was a significant reduction in seizures in the diet group compared to the control group and in the other study, they did not find a significant reduction but they did find a moderate reduction in seizures so we have mixed results.

The major barrier we face is the issue of compliance with ketogenic diet therapies in adults. The adult dietitian is essential for this process and can help tailor the diet to the individual. If the classic ketogenic diet is just not feasible, then we want to consider one of the modified, less restrictive diets as an alternative.

We did a study where we looked at improving the compliance on the ketogenic diet therapy in adult patients with epilepsy. We added a 4:1 ratio liquid formula to a Modified Atkins diet (MAD) during the first month of treatment to help boost ketosis compared to MAD alone. Seizures decreased in both study groups, however, participants that received the formula in the first month of treatment, were more likely to be on the diet after 6 months.

Lipid profiles in adults on ketogenic diet therapies:

- In the first 3-6 months on a ketogenic dietary therapy (KDT), the LDL and total cholesterol typically go up with compliant patients and then tend to trend down on their own without us making any changes, and by a year they tend to be the same as what they were at baseline.
- In one study, we looked at the LDL particle size. If the particles are big and fluffy, they can't enter into the blood vessel walls easily. If the particles are small, they can enter the vessels and cause plaque build-up (atherosclerosis) and blood vessel wall stiffening which can lead to heart disease, heart attack, and stroke. We found that there were

more of the small particles in patients who had been on the diet for a year or more compared to patients not on a KDT.

- We looked at carotid artery health and that was not different between the two groups.
- We are not sure what to make of this and so are now doing a longitudinal study in patients with epilepsy to make sure this isn't a long-term concern.

Unanswered Questions

- How long should adults with Glut1 Deficiency stay on a ketogenic diet therapy?
- How do hormonal changes affect Glut1 Deficiency symptoms and what are the interactions between hormones and ketone bodies?
- What long term side effects should we be monitoring and how should we be doing it?

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Ketogenic Best Practices and Fine Tuning

Ketogenic Diet: high fat, low carbohydrate and adequate protein diet
It is measured in ratios of grams of fat to grams of protein and carbohydrates combined

Building a well-defined care team:

- **Physician:** you want to be sure that you are working with someone who is well-versed with the ketogenic diet and that they are working closely with the dietician.
- **Dietician**: work with someone who is a registered dietician and should be able to provide you with advice in fine-tuning the diet.
- **Caregiver:** can encompass a wide range of people. It can be useful to describe the diet to them as medicine to help ensure that the diet is followed strictly.
- **Social support network**: can include family and friends and the better people understand the importance of the diet, the greater the support can be.

Comprehensive pre-diet clinic:

- Discuss diet parameters
- Consider social implications
- Review current diet
- Review current medications
- Discuss dietary goals

Fine-tuning the ketogenic diet through routine clinic follow-ups:

- Lab work: carnitine, acidosis, triglycerides and cholesterol, blood ketones, miscellaneous vitamins and minerals
- Review other symptoms: constipation, dehydration, diet fatigue
- Optimize efficacy: finding balance, higher ratio for kids, increased ketones, intermittent fasting, ketone esters, salts, C7
- Make it practical: tailor-made, time efficient, personalized
- Monitor growth: calories for growth, healthy weight maintenance
- Review any social implications you anticipate occurring

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Influences on Ketosis

The science and application of nutritional ketosis has been exploding. We have a study at Duke University where we have subjects that are on a ketogenic diet, they exercise and are exposed to very high levels of oxygen that push them to a seizure. We look at the latency to that seizure with and without the ketogenic diet and we also use a ketone supplement.

Our research has demonstrated that ketones really do influence many different parameters that are important and help preserve metabolic homeostasis from the cells to the organs. Many factors can influence glucose and ketone production and utilization.

When you measure your blood ketone levels, the ketones represent a source of energy that your cells are using, especially your brain and your heart. Exercise will lower your blood ketone levels in a similar way to how it lowers your blood glucose levels. We are using that energy as a source of fuel so if your ketones are low, especially after exercise, that may indicate that you are a good ketone utilizer. You are producing ketones and using them and that is dynamically changing.

Things that influence ketosis

- Your state of calorie restriction- if you are restricting calories you are more likely to lower blood glucose but also elevate your ketone levels
- The ratios and types of food used in the ketogenic diet
- Hormones
- Menstruation
- Exercise
- Stress
- Sleep
- Drugs
- Thyroid function
- Inflammation
- Infection
- Ketogenic fats
- Exogenous ketones