Glut1 Deficiency Foundation
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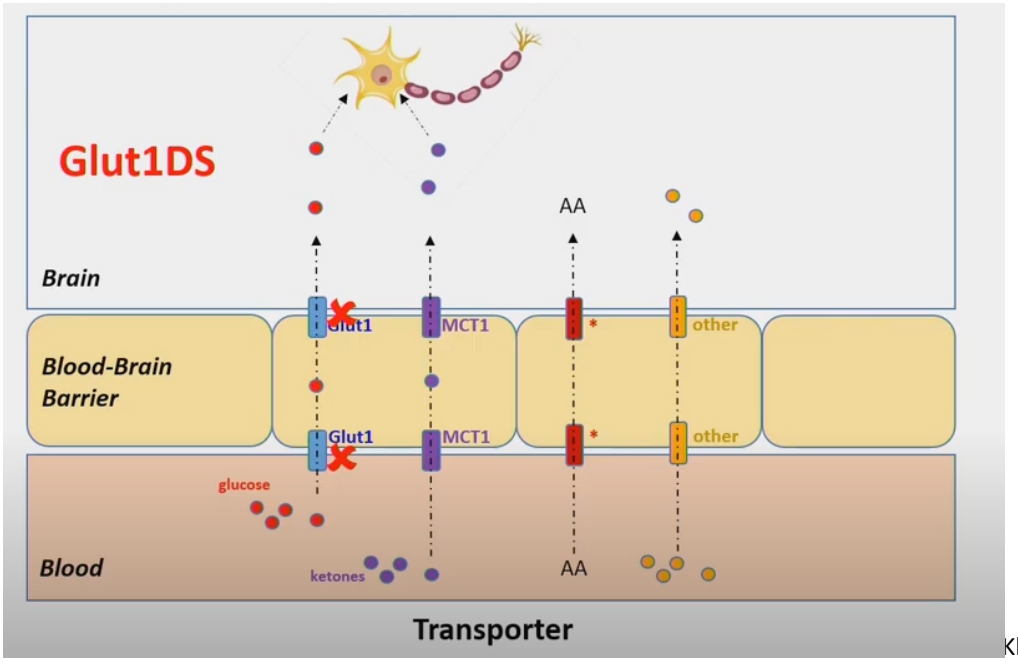
Glut1 Deficiency Insights

Glut1 Deficiency Syndrome - Scientific and Clinical Overview

Prof. Dr. Jörg Klepper

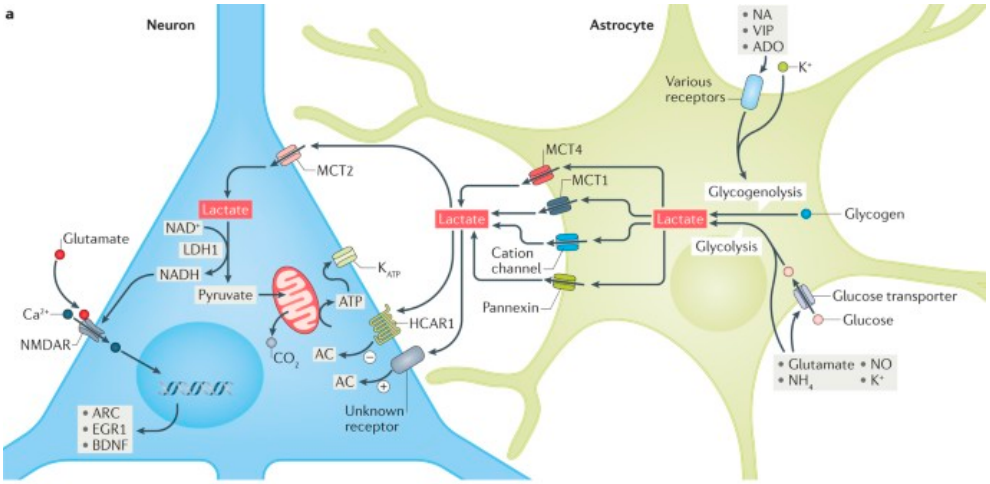
In 1991 the first Glut1 patient was described by Dr. De Vivo, they found a way to diagnose and treat patients using the Ketogenic Diet. Since then, there has been significant progress in the amount of information learned about the transporter and the condition. The symptoms are much more complex than originally thought, that not only mutations in the SLC2A1 gene can lead to the disease, but also that there are more treatments that can help our patients. In 2020 the first consensus paper was published, which is a big milestone for our community. It has helped to think about care and treatment for other patient populations, that were never thought of as having Glut1DS, such as adults and pregnant women.

The image below was presented by Dr. Klepper and summarizes how energy gets to the brain: Glucose (red circles) enters the brain from the bloodstream through the Glut1 transporter located in brain endothelial cells of the Blood Brain Barrier (BBB). When glucose is absent or Glut1 is absent, such as in Glut1DS, other transporters such as MCT1 can transport molecules that provide energy to the brain, such as ketones (purple circles). Then glucose or the products from glucose metabolism or ketones on the other hand, would provide energy to neurons. There are other transporters in the brain such as amino acid (AA) transporters.



Klepper, J. 2022

There are other molecules that are important in brain metabolism, such as lactate. Lactate is formed predominantly in astrocytes from glucose or glycogen in response to neuron activity signals. Lactate is then transferred to neurons to help supply their energy needs, and to provide signals that modulate neuronal functions, including excitability, plasticity and memory consolidation.



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Molecular interactions	Cellular mechanisms	Physiological processes
• MCTs • NADH/NAD+ • NMDARs • ATP • K_{ATP} channels • Lactate receptors • pH changes	• Neuronal plasticity • Axonal integrity • Neuronal excitability	• Learning and memory • Glucose sensing • Sodium sensing • Osmoregulation • pO_2 sensing • Ventilation • Arousal

Nature Reviews | Neuroscience

Magistretti, P Nature Reviews 2018

There are many different transporters that are involved in getting energy into the brain. Glucose plays many different roles in the brain, not just fuel for energy. The case of Glut1DS is much more than brain energy failure, the absence of glucose in the brain can lead to the disruption of other metabolic pathways that are necessary for brain function.

Questions raised on the scientific aspect of Glut1 DS:

- *Are there any other genes involved in Glut1 Deficiency syndrome?* Studies have shown that defects in other genes can lead to clinical and biochemical hallmarks that are generally associated with Glut1DS in the absence of mutations in the SLC2A1 gene. Could this explain the cases of patients with Glut1 DS symptoms but without a mutation on SLC2A1 gene? Which are those genes? There are still so many unknowns, but this opens a new avenue that could help improve diagnosis.

Key Clinical Symptoms for Glut1DS:

Paroxysmal eye–head movements, which are not seizures and are **an early symptom of Glut1DS in infancy.** This feature is now being taught to future neurologists, so that they learn to recognize it and take it into account as an early symptom of Glut1DS.

Another important symptom that some patients present is the **criss-cross gait**. This is a paroxysmal movement triggered by exertion or fasting. It is a lower-body **choreodyskinesia** causing the legs to intersect repeatedly producing irregular, random steps combined with some loss of balance. Patients experiencing this symptom, show a compensatory upper-body movements that help maintain balance. This symptom should help medical professionals suspect Glut1DS.

Long-term clinical course of Glut1DS

The clinical presentation of Glut1DS changes with age
Epilepsy: Is usually a symptom experienced by toddlers and young children
Movement disorder, especially dystonia: This symptom is experienced typically by adolescents and carried on into adulthood

Questions raised on the clinical aspect of Glut1 DS:

- *What happens in old age? Are there any symptoms that are typical of this stage?* There are still a lot of unknown aspects around the symptoms and care of adult patients

- *What is the reason that the ketogenic diet does not work for all the patients with Glut1DS? Which other treatments could help these patients?* There are studies regarding the ketogenic diet, how it works, why it doesn't work for everyone, how to supplement it and what can replace it in case it doesn't work. One of the things that was discussed was the ketone esters, which are supplements that may serve as a supplemental fuel for the brain without dietary restrictions, and they can achieve ketone plasma levels equivalent to the ketogenic diet. However, their daily values to reach those effects are very high and could cause a sodium overload.
- There's a need to establish newborn screening for early diagnosis and early treatment.

Each brain cell has a specific function. Understanding the way cells interact with each other, the metabolic pathways that are turned-on or off when cells interact or not, and taking in consideration the cells' location will help to have a better idea on how, when and where the absence of the Glut1 transporter affects these interactions.

Novel Biomarkers for Glut1DS

Prof. Dr. Michél Willemsen

What are biomarkers? The National Institutes of health defines biomarkers as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes (disease), or pharmacologic responses to a therapeutic intervention"
Example: CSF glucose

Why are they important*?* According to the FDA, biomarkers can tell how the body is doing in health and disease or how effective a treatment is. Having established biomarkers for a condition, for example Glut1 Deficiency, is integral to drug development. Biomarkers are measurable and they are used to measure the effect of investigational drugs on people during clinical trials. That's why it is important to establish new and better biomarkers for Glut1DS to improve diagnosis and to improve the success rate and the efficiency of drug development for this condition.

Dr. Willemsen analyzed CSF (Cerebrospinal Fluid) of patients with Glut1 deficiency and observed a significant reduction of three novel metabolites; one disaccharide (one molecule made of two sugars that are linked together), one trisaccharide (one molecule made of three sugars linked together) and and a mixture of two acids. The acids that he identified can serve as alternative substrates in an important metabolic pathway called **Pentose Phosphate Pathway (PPP)** which is a fundamental component of cellular metabolism. This metabolic pathway is

important to provide precursors or molecules necessary for the synthesis of nucleotides (blocks necessary to build DNA and RNA) and amino acids (blocks necessary to build proteins), as well as to provide molecules to defeat oxidative stress (oxidative stress can damage different molecules in your cells, such as DNA, proteins or lipids and this can eventually lead to a vast number of diseases over time).

The hypothesis Dr. Willemsen proposed is that low concentrations of the disaccharide and trisaccharide molecules reflect compromised O-glucosylation. ***Why is this relevant*?** O-glucosylation is a form of glycosylation. **Glycosylation** is a multistep process that takes place inside the cell; the purpose is to "decorate" proteins with sugar molecules that will enable proteins to function properly. O-glucosylation is a type of glycosylation.

Conclusions:

- Glut1 transporter does not only transport glucose, but it could also be needed to transport other sugars such as xylose and fucose into the brain, glycosylation may be compromised in Glut1DS due to a limited availability of multiple sugar molecules.
- Glut1 Deficiency syndrome is more than a disorder of brain energy failure.
- Three novel metabolites were identified in CFS of Glut1DS patients, which could be used as biomarkers of Glut1DS.

Current Research

Gene Therapy for Glut1 Deficiency Syndrome

Dr. Hitoshi Osaka

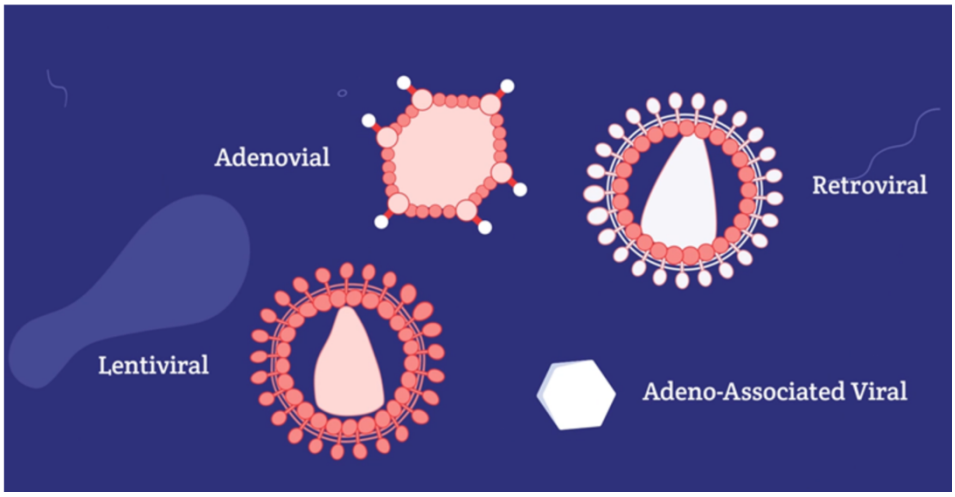
What is gene therapy?

Gene therapy is a technique that modifies a person's genes to treat or cure disease.
How does gene therapy work?
Gene therapy can work in different ways:

- Replacing a disease-causing gene with a healthy copy of the gene
- Inactivating a disease-causing gene that is not functioning properly
- Introducing a new or modified gene into the body to help treat a disease

Gene therapy can be performed by using different methods such as: Plasmids, viral vectors, bacterial vectors, human gene editing technology, patient-derived cellular gene therapy

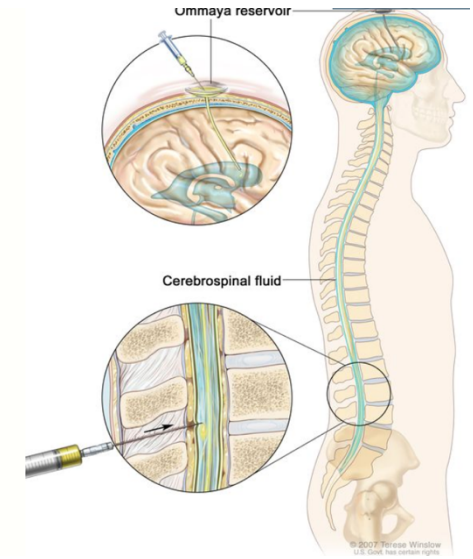
products. One of the most commonly used vectors for gene therapy are viral vectors. Virus have the ability to deliver genetic material into cells. Scientists have taken advantage of this characteristic to develop viral vectors that have been modified to inhibit their capability to cause disease and instead, carry a gene of interest to deliver it into human or any other animal cell for treatment.



Viral vectors used for gene therapy. ASGCT.org

Dr. Osaka is using a viral vector, an adeno-associated virus (AAV) as a vehicle to transport the Glut1 gene (SLC2A1).

His lab first performed experiments in mice to determine which route to use to deliver the vector. They tested the intraperitoneal and intrathecal routes of delivery. Next, they studied the expression or presence of the Glut1 protein in mice brain and determined which cells in the brain were expressing or producing the protein. Their results showed that mice deficient in Glut1 and treated with this vector using the intrathecal route, expressed Glut1 protein in different regions of the brain and in different cell types, such as endothelial cells and neurons among others. To study the possibility of translating this treatment into humans, Dr. Osaka's lab tested the delivery of this vector into pigs' using the intrathecal route.



Intrathecal route - Cancer.gov

Results in pig studies showed expression of Glut1 protein in different areas of the brain as well as in different cell types including endothelial cells and neurons.

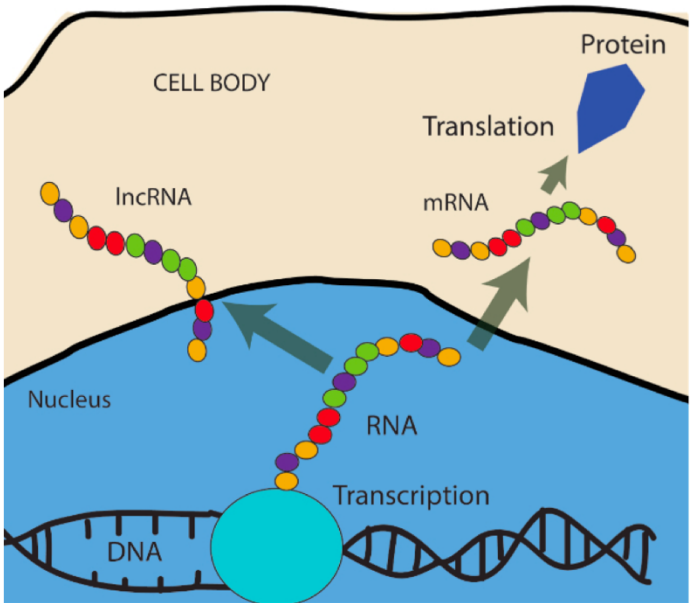
Dr. Osaka will start single center, open label Phase I/II clinical study in Japan in 2022-2023. The objective of this study is to determine the tolerability, safety and efficacy of intrathecal administration of gene therapy product in patients with Glut1 Deficiency. This study will be first conducted in adult patients.

A novel Glut1 regulatory element

Dr. Umrao Monani

There are molecules whose function is to regulate the expression of a gene. One of these molecules is regulatory RNAs. There are two types of these RNA molecules: microRNAs and long non-coding RNAs (lncRNA). Dr. Monani and his lab explored the possibility of finding long non-coding RNA molecules that could be regulating the expression of the Glut1 gene (SLC2A1).

What are long non-coding RNAs (lncRNAs)? lncRNAs are defined as RNAs longer than 200 nucleotides that are not translated into functional proteins. Studies done over the past years show that lncRNAs are widely expressed and have key roles in gene regulation.



Dr. Monani's lab found a long non-coding RNA located next to the human SLC2A1 gene. Their studies in cells showed that when this lncRNA is absent, the expression of the Glut1 protein decreases and when it is overexpressed, the expression of the Glut1 protein increases as well. Their results in mice showed that this lncRNA is highly expressed in central nervous tissues especially in the cortex of the brain.

In addition to these studies, Dr. Monani's lab has created a humanized mouse model of Glut1DS. which carry the human SLC2A1 gene as well as the lncRNA that was previously mentioned. This model will be key for future drug development experiments.