Pre-clinical assessment of GLUT1 Deficiency Syndrome high-throughput screen lead compounds.

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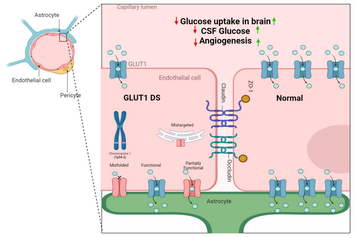
ABSTRACT

Haploinsufficient disorders involving mutations of a single allele, such as GLUT1 DS, are in essence dosage disorders, caused by pathologically reduced protein levels. In such cases, interventions increasing protein levels, including activation of the wild-type allele, may confer clinical benefit. In this regard, the SLC2A1 gene is known to be inducible, indeed various drugs have been shown to increase GLUT1 expression. Given that the concentrations needed to increase GLUT1 when tested in vitro in several cell lines often exceed that which is pharmacologically attainable, we propose the following research program to identify new therapeutic leads.

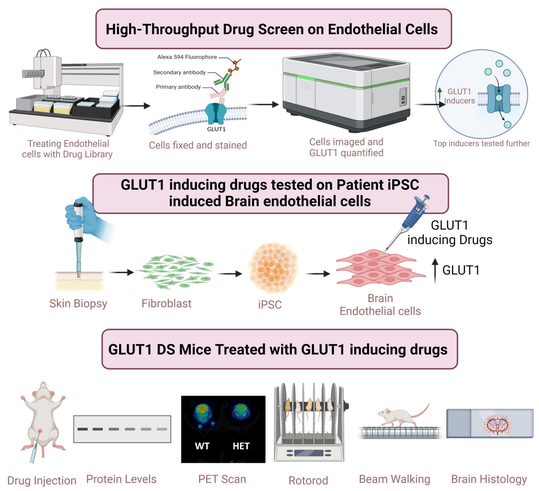
A screen assessing the impact of 800 FDA-approved drugs, as well as several GLUT1 inducing compounds, gleaned from the literature, on GLUT1 levels in wild-type endothelial cells (SLC2A1+/+) was undertaken. The top-inducing drugs and small molecules found in the screen shall be further validated in both in vitro and in vivo disease models. Fibroblasts from two GLUT1 DS patients' skin biopsies are being reprogrammed to induced pluripotent stem cells (iPSCs) and then further differentiated into induced brain endothelial cells (iBECs). The impact of the putative GLUT1 inducing compounds on glucose transport will be measured. We shall next test the impact of GLUT1 inducing drugs on heterozygous SLC2A1+/- mice, using both physiological and behavioural testing.

INTRODUCTION

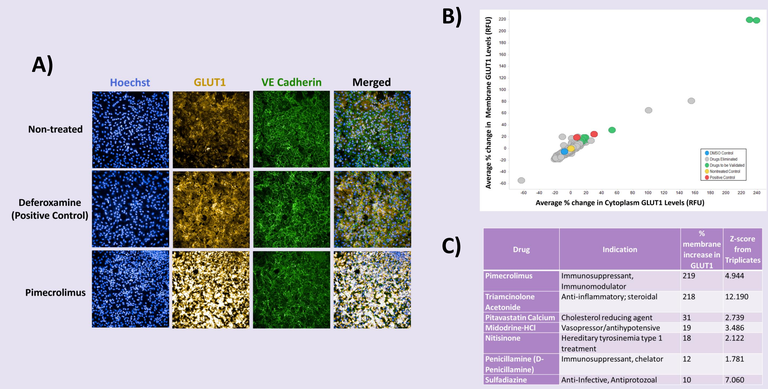


Glucose transporter 1 deficiency syndrome (GLUT1 DS) is an untreatable progressive autosomal dominant disorder caused by mutations in the gene encoding glucose transporter 1 (GLUT1), *SLC2A1*. Although a rare condition, it is estimated to impact hundreds of families in Canada and America alone. Approximately 90% of GLUT1 cases are caused by disabling de novo missense mutations in a single SLC2A1 allele. Given that endothelial GLUT1 is the predominant means of glucose transport across the blood-brain barrier (BBB), the resulting approximately 50% reduction in this critical glucose transporter results in decreased brain glucose levels. Currently there are no treatment and hence we are looking for a pharmacological approach to upregulate GLUT1

METHODS



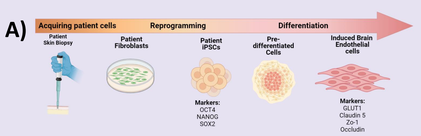
RESULTS



C)

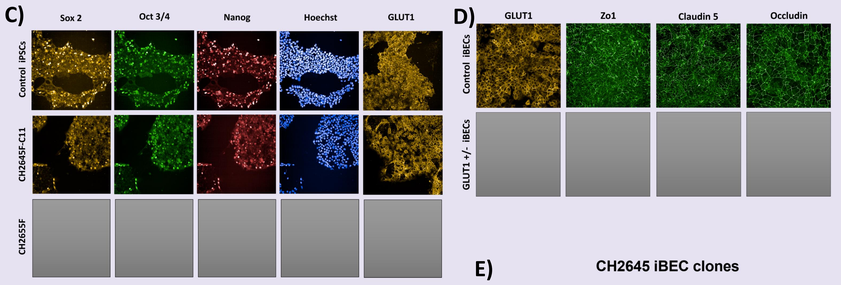
Drug	Indication	% membrane increase in GLUT1	Z-score from Triplicates
Pimecrolimus	Immunosuppressant, Immunomodulator	219	4.944
Triamcinolone Acetonide	Anti-inflammatory; steroidal	218	12.190
Pitavastatin Calcium	Cholesterol reducing agent	31	2.739
Midodrine-HCl	Vasopressor/antihypotensive	19	3.486
Nitisinone	Hereditary tyrosinemia type 1 treatment	18	2.122
Penicillamine (D-Penicillamine)	Immunosuppressant, chelator	12	1.781
Sulfadiazine	Anti-Infective, Antiprotozoal	10	7.060

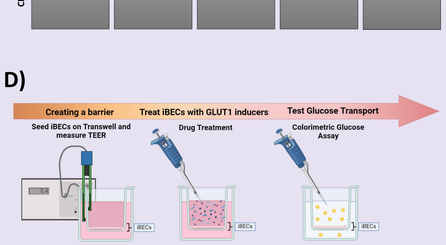
Figure 1: Screen-Well® FDA Approved Library II identifies several drugs upregulating GLUT1 levels in HUVEC cells. A) Sample staining of HUVEC cells post-treatment with Screen-Well® FDA Approved Library II for 24 hours. **B)** The average change (from triplicates) in GLUT1 levels in the cytoplasm and membrane of the HUVEC cells was quantified in RFU or relative fluorescence units using immunofluorescence microscopy. With each point representing a different treatment. Raw data was collected using Columbus and Z-scores calculated using sample mean. Drugs were eliminated based on 3 criteria: <10% increase in GLUT1 on the membrane when compared to the non-treated cells, a z-score of >1.5, and a lack of long term clinical safety. **C)** The top GLUT1 inducing drugs meeting all three criteria.



B)

Sample ID	Sex	Age at sample collection	Mutation	Stage of Differentiation
CH2645	F	4	**Pathogenic Splice Site Variant (c.972+3_972+6del (p.?))**	iPSC clones characterized and differentiation being optimized
CH2655	M	10	**Pathogenic Splice Mutation (c.517-2A>G)**	iPSC clones collected and need to be characterized





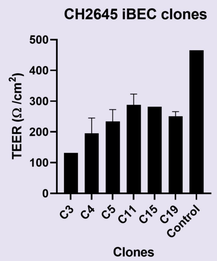


Figure 2 : GLUT1 DS Patient derived fibroblasts are reprogrammed into induced Pluripotent Stem Cells (iPSCs) and differentiated into induced Brain Endothelial Cells (iBECs). A) Steps to reprogramming and differentiation of patient fibroblasts and markers at each stage. **B)** Patient samples collected with their respective mutations. **C)** Control and Patient iPSCs stained with iPSC markers . **D)** Control iBECs stained with brain endothelial markers. Patient iBECs upcoming. **E)** Steps for upcoming barrier testing. **F)** Baseline TEER results for several clones of CH2645 patient iBECs compared to control iBECs, measured after seeding iBECs on semipermeable membrane of the insert as a measure of barrier integrity.

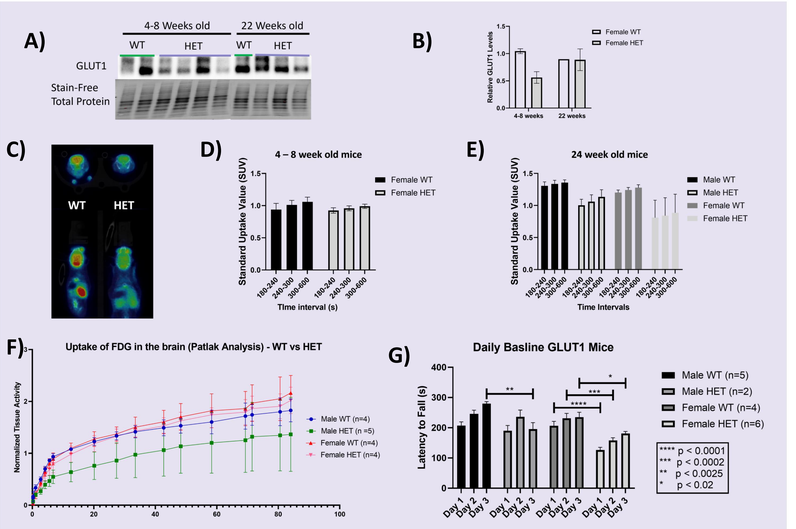


Figure 3: Baseline comparison of WT (SLC2A1$^{+/+}$) vs HET, (SLC2A1$^{+/-}$) mice. A and B)GLUT1 protein levels in the cortex of the brain in female mice 4-8 weeks and 22 weeks **C)** Representative image of the uptake of FDG as measured by PET scan in WT and HET mice. **D-G)** Mice were injected with 150-250 uCi of FDG via tail vein catheter under isoflurane and scanned for 1 hour using a microPET scanner. **D and E)**SUV of the brain between 180-600 sec in WT vs HET 4-8 week old and at 22 weeks of age respectively **F)** and the respective Patlak. **G)** Baseline comparison of WT vs HET mice on the rotarod test at 22 weeks of age.

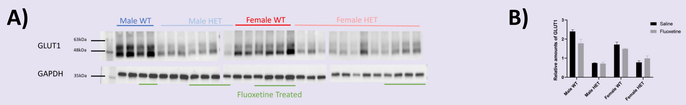


Figure 4: GLUT1 levels in the cortex of the brain after a 5 day treatment with fluoxetine at 10mg/kg. A) Normalized to GAPDH. B) No significant changes when comparing saline vs fluoxetine .

Conclusion and Future Directions

- **Further Validation:** Testing of top GLUT1-inducing compounds in patient-derived cells and animal models.
- **Expanded Screening:** Screen additional drugs to find more potential GLUT1 inducers.
- **Mechanistic Insights:** Investigate the pathways by which identified compounds upregulate GLUT1 expression for targeted therapy development.
- **Further Validation:** Continue in-depth testing of top GLUT1-inducing compounds in patient-derived cells and animal models.
- **Clinical Trials:** Initiate trials with the most promising drugs in GLUT1 DS patients.

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