Uncovering Glut-1 Deficiency Syndrome Mechanisms and Therapeutic Targets Using Patient-Derived Brain Organoids

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Glut-1 Deficiency Syndrome (Glut-1DS) is a rare autosomal dominant neurological disorder caused by heterozygous mutations in the SLC2A1 gene, leading to impaired glucose transport across the blood-brain barrier (BBB). Since glucose is the primary energy source for the brain, Glut-1 haploinsufficiency results in severe neurological symptoms, including epilepsy, developmental delay, intellectual disability, and movement disorders. Currently, the ketogenic diet is the only available treatment, but while it mitigates seizures in some patients, it fails to address other neurodevelopmental deficits. Understanding Glut-1DS pathophysiology and identifying novel therapeutic targets remain major challenges due to the lack of physiologically relevant human models.

To address this, we propose to generate patient-specific brain organoids from induced pluripotent stem cells (iPSCs) derived from peripheral blood mononuclear cells (PBMCs) of Glut-1DS patients. Brain organoids provide an advanced 3D human model that recapitulates early neurodevelopment, offering unique insights into disease mechanisms. As an initial step, we have successfully established brain organoids from healthy control iPSCs, confirming proper neural differentiation, cortical layer formation, and BBB development. Our next objective is to generate Glut-1DS patient-derived organoids and compare them with healthy controls to identify disease-specific phenotypic alterations.

Our experimental approach includes:

iPSC reprogramming and differentiation: we will generate iPSCs from Glut-1DS patient PBMCs and differentiate them into neural progenitors, astrocytes, and endothelial cells.

Brain organoid generation and characterization: patient-derived organoids will be evaluated for neural differentiation markers, structural integrity, and metabolic function using immunohistochemistry, qRT-PCR, and transcriptomic profiling.

BBB modeling and functional assays: given the critical role of Glut-1 in endothelial cells, we will establish a patient-specific BBB model using iPSC-derived endothelial cells co-cultured with astrocytes and brain organoids. We will assess permeability, Glut-1 expression, and junction protein integrity.

Comparative analysis and biomarker identification: metabolic profiling, electrophysiological recordings, and single-cell RNA sequencing will be performed to identify disease-specific alterations and potential therapeutic targets.

Generating brain organoids directly from patients offers a unique and valid human 3D model that enables detailed functional studies of Glut-1DS pathogenesis and potentially provides an easier way to screen for new therapies.