"Why We Need Glut1 Deficiency Pig Models for Research"

GLUT1 DEFICIENCY SUMMIT 2024

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Abstract

The research aims to identify the disease locus for GLUT1 Deficiency (G1D) by leveraging patient EEGs, fMRI, and 18-FDG PET scans. Prior mouse model studies suggested the seizure locus in layer V parvalbumin-positive inhibitory neurons projecting to the thalamus. However, mouse models have limitations due to developmental differences and indirect glucose absorption measures of human methodologies. To address these, a pig model is proposed to enable detailed in-vivo neurophysiology and metabolic measurements from multiple brain regions, more accurately identifying the disease locus.

Why the Pig Model?

Pigs offer several advantages for studying GLUT1 Deficiency:

- Anatomical Similarities: Pigs share structural and physiological similarities with humans, making them suitable for extrapolating dosage, administration routes, and treatment outcomes.
- Ease of Procurement: Pigs are more accessible and cost-effective compared to other non-human primates.
- Developmental Benefits: Pigs mature quickly (6-7 months) and have a short gestation period (~112 days) with large litter sizes (~10 piglets), facilitating rapid and large-scale studies.
- Longer Lifespan: Their longer lifespan (15-20 years) allows for extended longitudinal studies of long-term effects.
- Large Brain Size: The larger brain size enables detailed neurophysiological and metabolic studies that are challenging in smaller animals like mice.

Methods of model development

Various techniques exist and have been employed in the past to genetically modified pigs exhibiting various phenotypes of diseases. Techniques includes:

- Microinjection: Injecting DNA/RNA/Protein into the pronucleus or cytoplasm.
- Sperm Mediated Gene Transfer (SMGT): DNA injection into oocytes during fertilization.
- Somatic Cell Nuclear Transfers: Transferring somatic nuclei into mature denucleated oocytes.
- Gene Targeting: Using CRISPR/Cas9, Zinc-finger nucleases (ZFNs), and Transcription activator-like effector nucleases (TALENS) for specific gene edits.

Our Success

- Surgical Expertise: We have established methods for performing brain surgeries and identifying brain vasculature for effective understanding of blood supply.
- Electrophysiology Apparatus: We have built noise-free electrophysiology apparatus and stereotactic frames for precise electrode insertions.
- Characterization of Anesthesia Effects: We have deeply studied the effects of anesthesia agents and continuously monitored brain physiological parameters such as tissue oxygenation, intracranial pressure, and temperature.
- Neurophysiological Comparison: Anesthetized pigs have exhibited brain neurophysiology similar to awake humans, unlike awake mice whose data significantly differ from humans. This makes pigs a more accurate model for human brain studies.

Results and Conclusion

Preliminary results indicate that the pig model allows for detailed regional metabolic and neurophysiological data, overcoming the limitations of mouse models. This research aims to correlate glucose presence with metabolic and neural activity changes, closely resembling patient-based research.

Future Directions

We suggest that a collaborative grant application to the funding agencies would support the development and maintenance of the pig model colony for ongoing and future testing. This would significantly advance the understanding and treatment of GLUT1 deficiency, aligning with current clinical and scientific goals.