GAD65+ Autoimmune Epilepsy Shows Evidence for Hippocampal Hyperexcitability and Inflammation: A Case Study Using HD Spatial Transcriptomics by 10X Genomics

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Objective

To investigate the pathological mechanisms underlying GAD65+ autoimmune epilepsy in a patient with refractory epilepsy, specifically focusing on observed neuronal hyperexcitability in the hippocampus using the novel HD Visium technology.

Background

- Inhibitor signaling in the brain relies on GABA, catalyzed by glutamic acid decarboxylase-65 (GAD65).
- Low-titer GAD65 disease represents a diverse category of diseases including limbic encephalitis and autoimmune epilepsy.
- GAD65 autoimmune epilepsy is a rare but important cause of refractory and recurrent seizures that are thought to be underdiagnosed among epilepsy cases.
- The proper diagnosis can be missed without adequate antibody testing and patients are often refractory to typical antiseizure medications.

Methods

- A 21-year-old female with refractory seizures underwent right temporal lobectomy for seizure control. She was later found to have GAD65+ autoimmune epilepsy and developed recurrent epilepsy in the contralateral hippocampus shortly after resection.
- Hippocampal histopathology staining included H&E, inflammatory markers, GAD65/67, Glycine receptor (GlyR)-alpha-3 subunit, and parvalbumin, and tissues were analyzed by a board-certified neuropathologist (SG).
- Spatial transcriptomics was performed using the HD Visium platform, profiling gene expression across various regions of the hippocampus from the biopsy tissue.
- HD Visium captures a 6.5 x 6.5 mm area with a continuous lawn of oligonucleotides arrayed into millions of 2 x 2 µm barcoded squares, allowing spatial assessment of gene expression.

Results

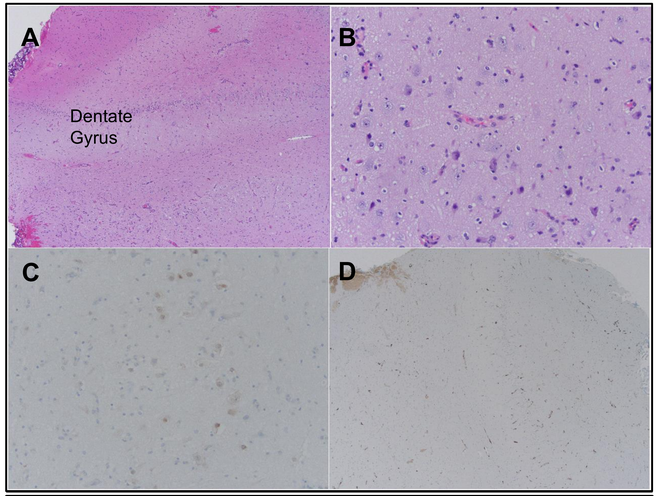
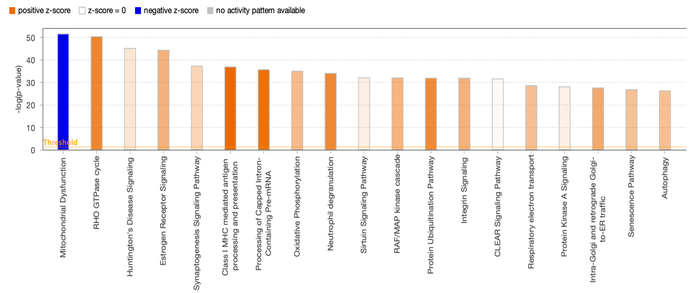


Figure 1: Histopathology of the Hippocampus in GAD65 Associated Epilepsy
Stains of interest include GFAP (**A**), NeuN (**B**), CD68 (**C**) and CD163 (**D**). **A** Demonstrating increased perineural inflammation and heavy reactive gliosis. **B** Demonstrating similar loss of neurons. **C, D** Demonstrating scattered microglial cells throughout the hippocampus.

Table 1: Key Pathways of Transcriptomic Dysregulation in GAD65 Epilepsy Compared to Control



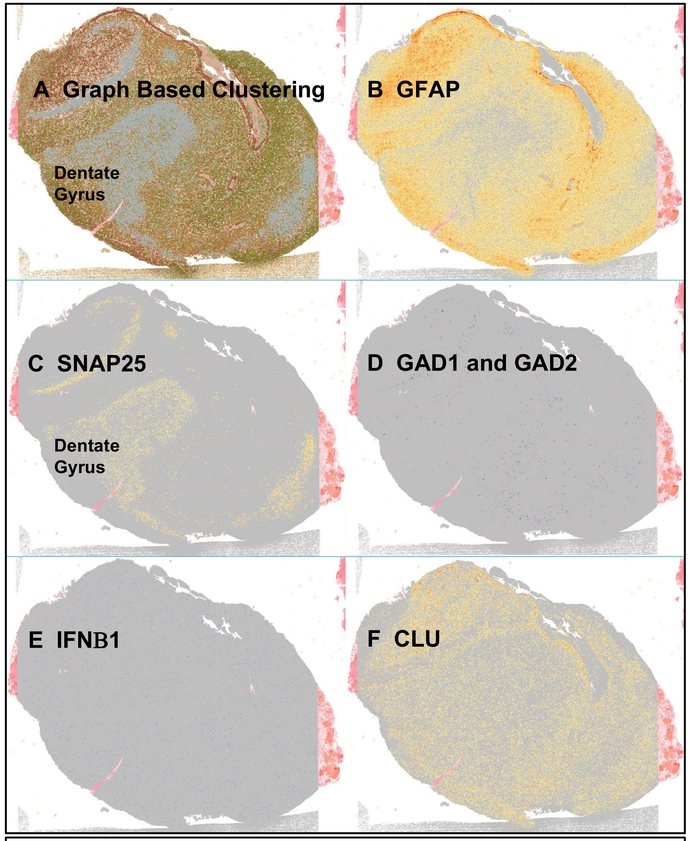


Figure 2: HD Visium Spatial Transcriptomics of the Hippocampus in GAD65-associated Epilepsy
Images by BioTuring for 10X Genomics HD Visium Spatial Transcriptomics. **A** Graph Based Cluster Image of hippocampus with light blue representing the neuron cluster. **B-F** Gene expression profiles including various degrees of positive regions.

Abbreviations: GFAP, glial fibrillary acidic protein; SNAP25, synaptosomal-associated protein 25; GAD, glutamate decarboxylase or GAD1 (aka GAD67); IFNB, interferon beta; CLU, clusterin.

Discussion

- Hippocampal tissue showed diffuse T cell inflammation and heavy reactive microgliosis, as well as loss of pyramidal neurons in CA1 and CA4.
- Spatial transcriptome profiling identified several relevant canonical pathways such as significant inhibition of GABA and overexpression of glutamate, overall generating excitotoxicity and enriching for oxidative stress and cellular stress response pathways.
- The Class I MHC-mediated antigen processing canonical pathway was also significantly increased compared to normal control. Interestingly, the KEGG Pathway Analysis highlighted several viral entities following pseudo-bulking of the data. IFNB1 gene expression is an example of this pathway activation.
- This transcriptomic analysis suggests widespread dysregulation across multiple pathways relevant to GAD65 epilepsy.
- This case highlights the role of GAD65 autoantibodies in disrupting GABAergic signaling and promoting neuronal hyperactivity and inflammation within the hippocampus.
- The use of HD Visium allowed for detailed spatial gene expression profiling, which underscores the importance of understanding the molecular underpinnings of autoimmune epilepsy and may inform the development of targeted therapies.

References

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