Effects of deafness on central neural cell chemistry: a spatial transcriptomic analysis of the auditory cortex and hippocampus in a mouse model of congenital deafness

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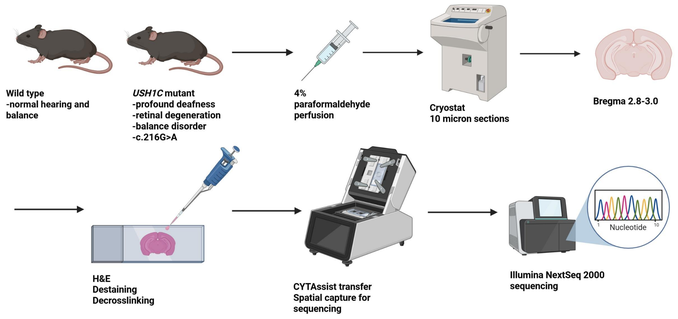
Introduction

Objective: According to the World Health Organization (WHO), approximately 10 million new cases of dementia are diagnosed annually – a number expected to rise with an aging global population. Hearing loss is a significant modifiable risk factor, accounting for an estimated 8% of dementia cases. While clinical interventions such as hearing aids and cochlear implants have been shown to reduce dementia risk, the underlying molecular mechanisms linking auditory deprivation to cognitive decline remain poorly understood. We used a mouse model of Usher syndrome based on the human 216A mutation to investigate how congenital deafness alters gene expression in the auditory cortex and hippocampus – regions critical for auditory processing and memory. The USH1C gene with the c.216G>A mutation causes human Usher syndrome, the leading genetic cause of combined deafness and blindness. Because auditory circuitry exhibits activity dependent development, we hypothesize that genes associated with synaptogenesis and neural plasticity in the auditory cortex will be dysregulated in congenitally deaf mice compared to wild-type controls.

Methods: General



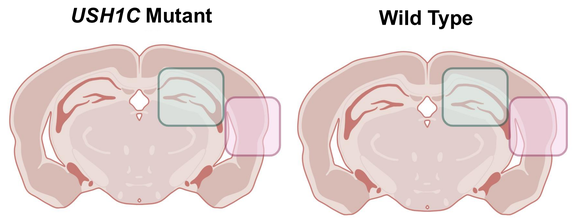
We employed spatial transcriptomics (10xGenomics Visium) to analyze brain tissue from wild-type (WT) and USH1C mutant (congenitally deaf) mice. Brains were perfused with paraformaldehyde (PFA), cryosectioned at 10 µm thickness (bregma +2.8 to +3.2 mm), and processed using the CytAssist-enabled Visium protocol. Following H&E staining, decrosslinking, destaining, and CYTAssist enabled transfer onto Visium Spatial Gene Expression slides (10x Genomics), which automates tissue processing and image capture, spatially barcoded mRNA was captured, reverse-transcribed, pooled, normalized and sequenced on an Illumina NextSeq 2000 platform using a NextSeq 2000 P4 (100 cycles) flow cell according to manufacturer's recommendations. Data was analyzed using the10xGenomics Space Ranger pipeline.

Sequencing: Tissue sections were transferred onto the fiducial frames of a Visium Spatial Gene Expression slide (PN-2000233, 10x Genomics, Pleasanton, CA) using the Visium CytAssist. The tissues underwent spatial barcoding, and cDNA library was generated from the tissue mRNA as outlined in the Visium Spatial Gene Expression User Guide (CG000239, 10x Genomics). The mRNA transcriptome was mapped to a 55 µm spatial resolution. The mRNA library was sequenced using a NextSeq 2000 platform. Sequencing outputs were processed using 10x pipeline SpaceRanger.

Computational Methods: Space ranger outputs were loaded as a Seurat object in R using Load10x_Spatial. Quality control was performed by removing all spots with an nCount_Spatial less than 100. Normalization was performed using SCTransform. Principal component analysis and dimensionality reduction were performed using RunPCA, FindNeighbors, FindClusters and RunUMAP with a resolution of 0.5 and principal component 1:30. Differential expression testing was performed using Seurat FindMarkers using a Wilcoxon rank sum test. P-values were adjusted for multiple testing using the Bonferroni testing.

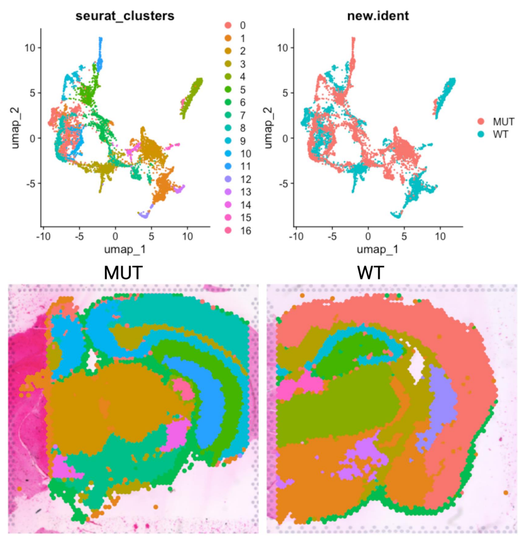
Methods: Anatomical Clustering Confirmed

Illustration of mouse coronal sections at ~2.7-3.2 Bregma



comparison of gene expression fold change between mutant and wild-type **hippocampus** and **auditory cortex**

Two Regions of Interest



Regions of interest are compared. Gene expression clustering in the regions is confirmed.

Fig.1: Seurat clustering between wild type and mutant. UMAPs (top) depicting cell clusters determined using Seurat workflow (left) and by sample (right). Clusters visualized spatially on each image (bottom).

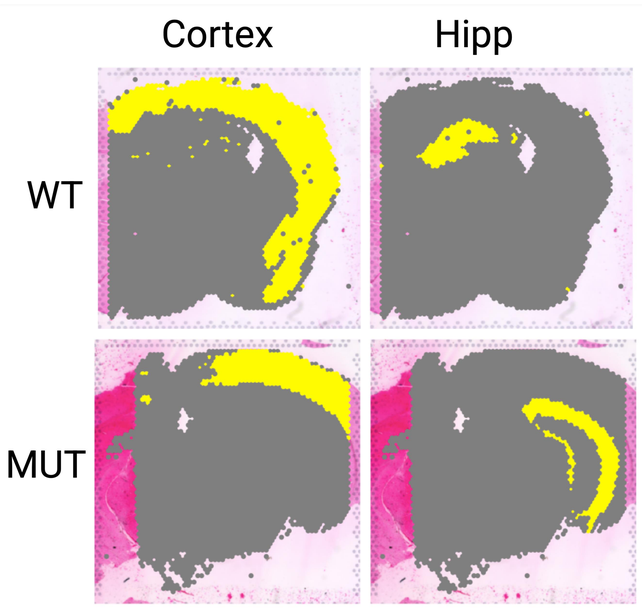


Fig.2: Defining Visium spots in anatomical regions. Highlighted cells for anatomical regions of interest based on Seurat clustering in WT (top) and MUT (bottom).

Results

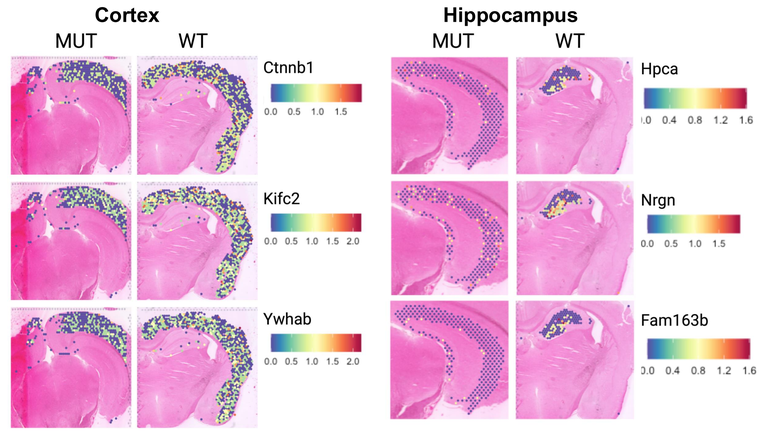


Fig.3: Differentially expressed genes of interest. Gene expression profiles depicted spatially between MUT and WT in the cortex (left) and hippocampus (right). Differential expression testing was performed using Seurat FindMarkers function following SCT normalization. All genes displayed had a pval < 0.05 after correcting for multiple testing and a fold change > |0.5|.

Cortex

Gene Abbreviation	Gene Name	Function	Expression change
Hpca	Hippocalcin	Protein: hippocalcin, a calcium-binding protein. Linked to: neurodevelopmental delay, intellectual disability, infantile seizures, chorea, and cognitive decline.	Downregulated
Nrgn	Neurogranin	Protein: neurogranin, exclusively in the brain and is critical for synaptic plasticity and memory. Associated with Schizophrenia, Alzheimer's, TBI	
Fam163b	Family with sequence similarity 163 member B	neuroregulatory gene that may play a role in the function of synaptic proteins	

Hippocampus

Gene Abbreviation	Gene Name	Function	Expression change
Ctnnb1	catenin beta 1	Protein: beta-catenin. Cell adhesion. involved in intellectual disability, autism, and schizophrenia.	Downregulated
Kifc2	kinesin family member C2	Microtubule and axonal and dendritic transport. Dysregulation leads to neuro-tumors, neurodegenerative disease, and psychiatric illnesses	
Ywhab	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Beta	Mediates signal transduction. Dysregulated expression in glioblastoma and schizophrenia	

Results: Cell clusters were annotated using Allen Brain Atlas markers, and UMAPs were generated. There was significant differential gene expression across the two mice types. Tables show top six genes with downregulated expression as compared to wild-type mice.

Conclusion

This study provides a molecular framework for understanding how auditory deprivation may contribute to cognitive decline and dementia risk and aims to provide critical insights into the molecular consequences of congenital deafness on central neural cell chemistry, particularly within brain regions vital for auditory processing and cognition. The findings suggest that congenital deafness induces transcriptional reprogramming in the auditory cortex and hippocampus. This study underscores the utility of spatial transcriptomics in uncovering region-specific molecular changes underlying sensory deprivation and cognitive vulnerability.

Next steps: In addition to increasing sample size, the study will evaluate if gene dysregulation is reversed by comparing antisenseoligonucleotide (ASO)-treated USH1C mice with WT and Mutants. ASO treatment reduces relative expression of mutant harmonin protein in USH1C mice, leading to rescue of hearing, vision, and vestibular function (1). ASO-treated mice are predicted to show partial restoration of these gene expression patterns.

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References:
1. Lentz, J., Jodelka, F, Hinrich, A., McCaffrey, K., Farris, H.E., Spalitta, M., Bazan, N., Duelli, D., Rigo, F., Hastings, M. (2013) Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness. Nature Medicine doi:10.1038/nm.3106