Analysis of RNA Editing in Human NFT-bearing and NFT-free Neurons

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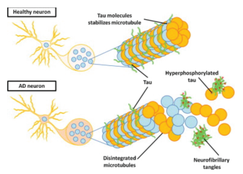
Abstract

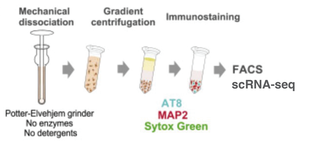
Hyperphosphorylated tau proteins aggregate into neurofibrillary tangles (NFTs), which are hallmarks of Alzheimer's Disease (AD). While a difference in RNA editing has been found between AD patients and controls, the link between RNA editing and NFTs remains unclear. Here, we analyzed single-cell RNA-seq data from 8 AD donors and called differential RNA editing sites (DEs) between NFT-bearing and NFT-free neurons using de novo RNA editing identification and REDIT testing for differential sites. We identified 64,087 DEs in excitatory neurons, 56% of which showed an increase in editing, and 7,661 in inhibitory neurons, 76% showed an increase. 50 DEs fall in protein-coding regions, including SORBS1, GRIK2, NAE1, and HMGA2, which are AD-relevant genes. To our knowledge, this study is the first to map global RNA editing profiles related to tau pathology, and the identified NFT-neuron-specific editing sites help pave the way to further understand mechanisms behind RNA editing's relevance to tauopathies.

Introduction

- Tau is a microtubule-associated protein that stabilizes microtubules in healthy neurons.
- Hyperphosphorylation of the tau protein leads to formation of NFTs, a hallmark of Alzheimer's Disease.
- Tau aggregation can induce neuroinflammation, which may influence the expression of RNA editing enzymes.[1]
- RNA editing modifies protein coding sequences, splicing, and other RNA features, and differs between AD patients' and healthy brain tissues.[2]
- Whether RNA editing differs between NFT-bearing and NFT-free neurons within the same patient is unknown.
- **Hypothesis**: **There is a difference in RNA editing between NFT-bearing and NFT-free neurons**.



- We analyzed snRNA-seq data from a previous study that isolates NFT-bearing (AT8+) and NFT-free (MAP2+) neurons from the same Alzheimer's disease patients.[3]



Results

Identification of RNA editing sites in excitatory and inhibitory neurons

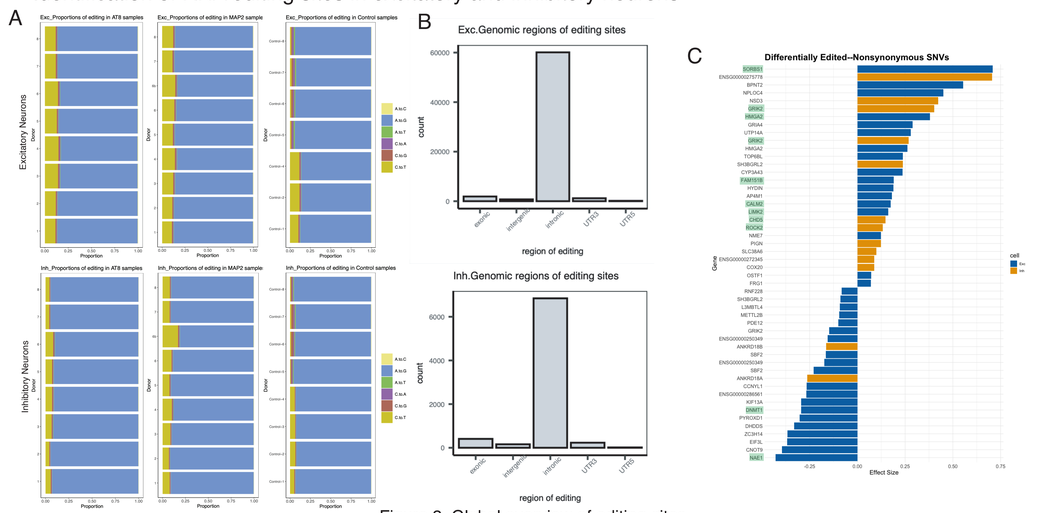
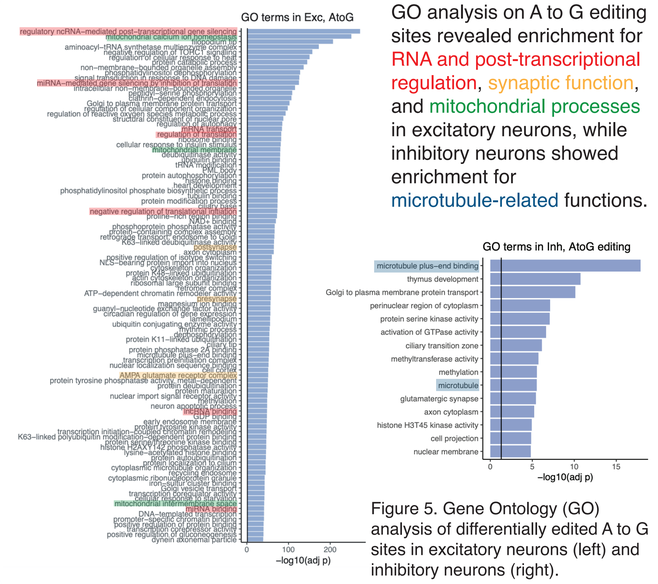


Figure 2. Global overview of editing sites.
(A) Proportion of editing types in excitatory (top) and inhibitory (bottom) neurons across NFT-bearing, NFT-free, and control samples. (B) Genomic regions of editing sites. (C) Genes associated with the differentially edited sites in protein-coding regions. Green highlight indicates known AD-relevant genes.



GO analysis on A to G editing sites revealed enrichment for RNA and post-transcriptional regulation, synaptic function, and mitochondrial processes in excitatory neurons, while inhibitory neurons showed enrichment for microtubule-related functions.

Figure 5. Gene Ontology (GO) analysis of differentially edited A to G sites in excitatory neurons (left) and inhibitory neurons (right).

There is a higher proportion of sites showing an increase in A to G editing in both excitatory and inhibitory NFT-bearing neurons, despite the decrease in ADAR2 expression.

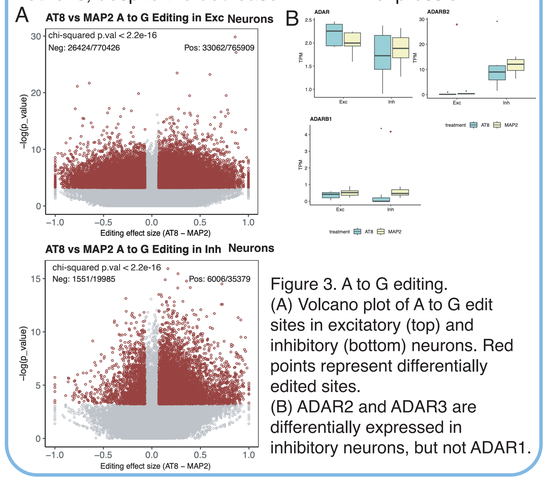


Figure 3. A to G editing.
(A) Volcano plot of A to G edit sites in excitatory (top) and inhibitory (bottom) neurons. Red points represent differentially edited sites.
(B) ADAR2 and ADAR3 are differentially expressed in inhibitory neurons, but not ADAR1.

A similar trend is observed in C to U editing; there is an increase in editing in AT8 neurons despite the decrease in gene expression levels of proteins in the APOBEC family.

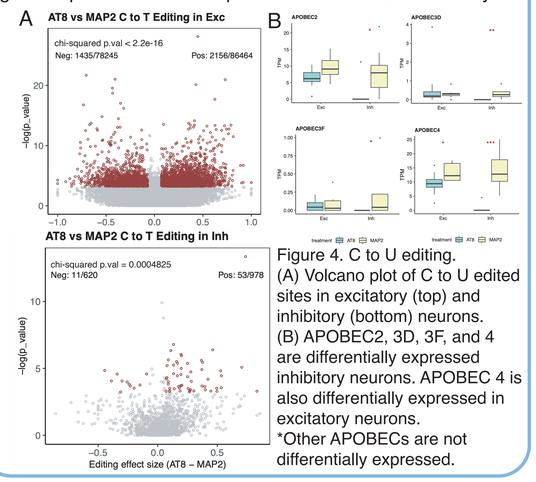
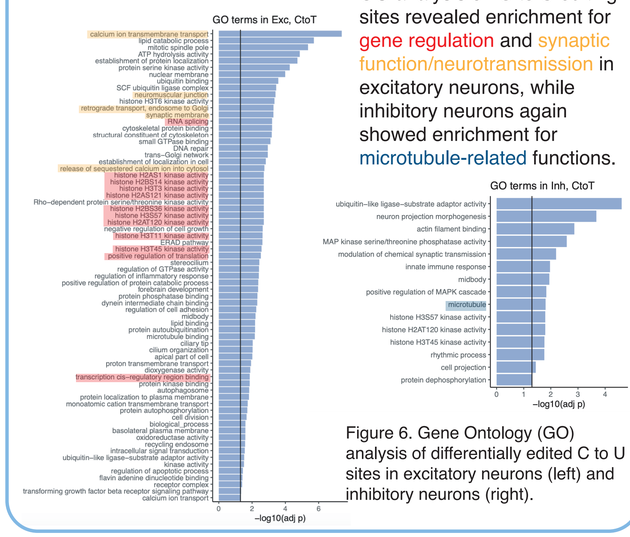


Figure 4. C to U editing.
(A) Volcano plot of C to U edited sites in excitatory (top) and inhibitory (bottom) neurons.
(B) APOBEC2, 3D, 3F, and 4 are differentially expressed inhibitory neurons. APOBEC 4 is also differentially expressed in excitatory neurons.
*Other APOBECs are not differentially expressed.



GO analysis on C to U editing sites revealed enrichment for gene regulation and synaptic function/neurotransmission in excitatory neurons, while inhibitory neurons again showed enrichment for microtubule-related functions.

Figure 6. Gene Ontology (GO) analysis of differentially edited C to U sites in excitatory neurons (left) and inhibitory neurons (right).

Methods

1. Read Alignment and Preprocessing

- Extract splice junctions with STAR
- Second pass mapping with CellRanger v9
- Hyperediting alignment

2. Clustering and Cell Type Assignment

- Cluster with Seurat v5
- Assign cell types with enrichment marker genes
- Remove doublets

3. Deduplication

- Remove PCR duplication effects on mapping
- Pooling cells per cell type per donor into pseudo-bulk bam files

4. RNA-DNA Differences Pipeline and Annotation

- Run RDD pipeline to identify RNA-DNA mismatches, editing ratio, and log-likelihood ratios
- ANNOVAR for variant function annotation
- UCSC genome browser for alu annotations

5. Differential Editing Analysis

- Run REDIT log-likelihood ratio test to find editing likelihood
- Filter for LLR p value < 0.05 and effect size > 0.05
- Calculate gene expression based on TPM
- Gene Ontology analysis

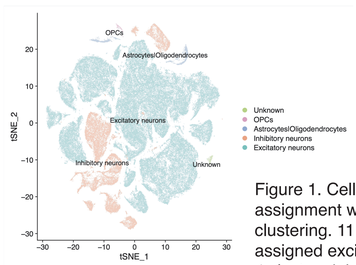


Figure 1. Cell type assignment with Seurat clustering. 11 clusters were assigned excitatory neurons, 4 clusters inhibitory neurons.

Discussion

- Our identification of thousands of differentially edited sites between NFT-free and NFT-bearing neurons, supporting our hypothesis that RNA editing differs between these cells.
- AD-relevant sites were among the differentially edited protein-coding regions, indicating that RNA editing may contribute to AD processes.
- Despite decreased expression in ADAR2 and APOBECs in NFT-bearing neurons, many sites showed an increase in editing ratio, suggesting alternate substrate concentration or the possibility of other RNA editing proteins.
- GO analysis reveal association between A to G and C to U editing and biological pathways related to AD, including synaptic transmission and microtubule function.
- Future work will examine the correlation between double stranded RNA and NFT-bearing neurons. We will also correlate NFT with neuroinflammatory signals.

References and Acknowledgements

Works cited

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