



An iPSC-derived forebrain organoid model to dissect the consequences of a risk variant in *TTC3*

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BACKGROUND

Utilizing whole exome sequencing, we previously identified a rare, nonsynonymous variant in the *tetratricopeptide repeat domain 3* (*TTC3*) gene that segregated in all 11 Alzheimer disease (AD) individuals in a non-Hispanic white late onset Alzheimer disease (LOAD) family (Kohli, Cukier, et al, *Neurol Genet.*, 2016, **Fig. 1**). This missense alteration, rs377155188 (p.S1038C), is predicted to be deleterious and is extremely rare in the gnomAD database (allele frequency=3.231x10⁻⁵). Studies have reported that cortical *TTC3* expression is reduced in LOAD patients and negatively correlated with the AD neuropathology.

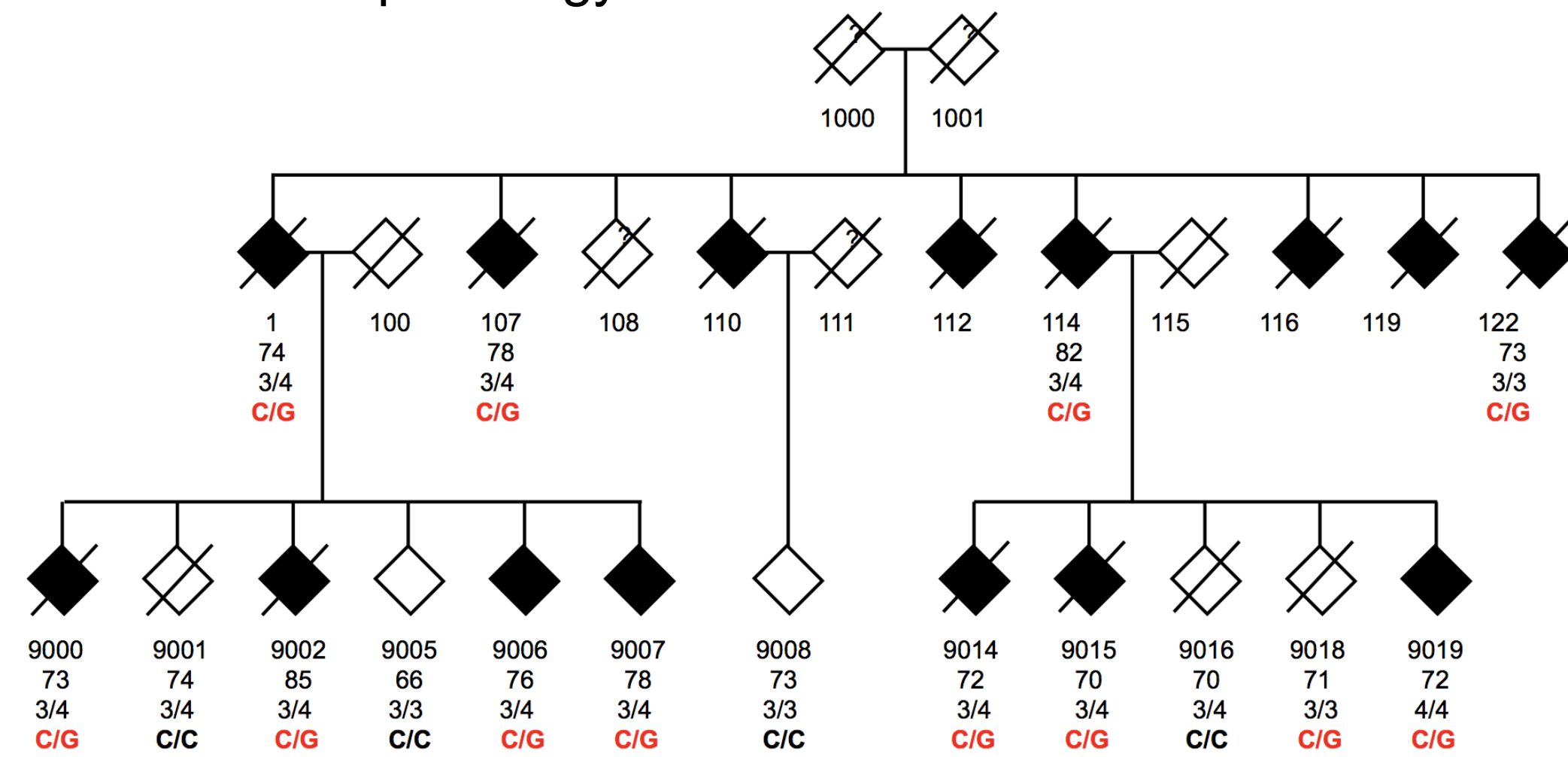


Figure 1. Family 1229. Pedigree with 15 affected individuals. The age shown is either the age at onset or the age at exam. The heterozygous *TTC3* variant (shown in red) was found in all 11 AD individuals that were sequenced.

HYPOTHESIS

To understand the mechanism by which the *TTC3* p.S1038C may contribute to LOAD risk, CRISPR/Cas9 genome edited induced pluripotent stem cells (iPSCs) were developed that were homozygous for the variant to examine cellular and transcriptional consequences in neuronal cells.

METHODS AND RESULTS

Generation of isogenic iPSCs
CRISPR/Cas9 genome edited induced pluripotent stem cells (iPSCs) homozygous for the *TTC3* variant p.S1038C were developed, UMi028-A-2, thereby generating an isogenic pair between the parental ASE-9203 unedited line and the edited line (Laverde-Paz, et al, *Stem Cell Res.*, 2021).

METHODS AND RESULTS

Differentiation into 2D neuronal cultures

The isogenic iPSCs were differentiated into forebrain neurons. Quantitative PCR analysis demonstrated that *TTC3* levels were decreased in edited iPSCs, as well as day 30 neuronal precursor cells (NPCs) and day 70 neurons (**Fig. 2**).

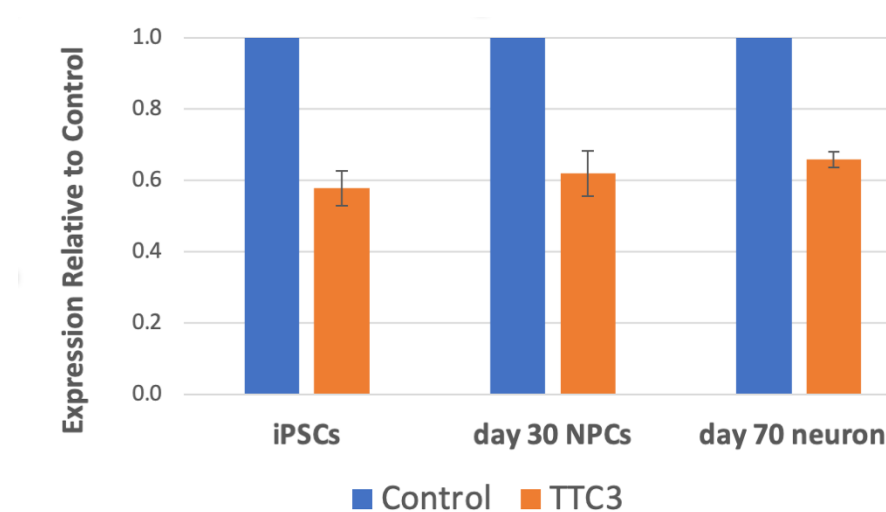


Figure 2. *TTC3* expression is lowered by ~40% edited cells.

Differentiation into 3D organoid cultures

The isogenic iPSCs were differentiated into organoids using the STEMCELL Technologies STEMdiff Dorsal Forebrain Organoid Differentiation kit (**Fig. 3**). The organoids were grown to day 130, fixed for staining via immunofluorescence and quantified (**Fig. 4**), as well as dissociated and evaluated via single cell RNA-sequencing (scRNA-seq) utilizing the 10X Genomics platform and Illumina sequencing. For scRNA-seq, five organoids were collected per pool, with two pools were collected with either organoids from the edited or the unedited cell line. We targeted 5k cells per pool/50k reads per cell.

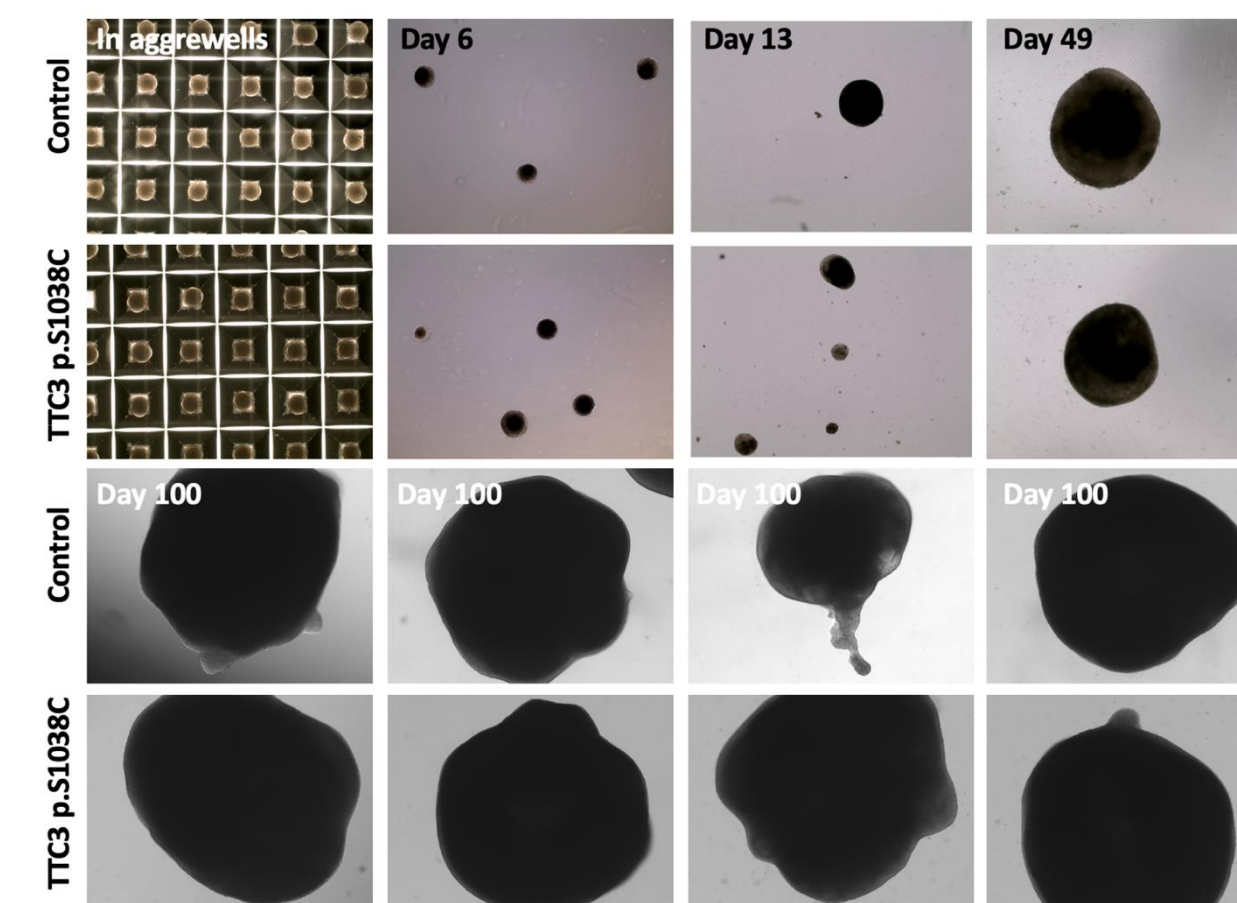


Figure 3. Development of 3D organoids

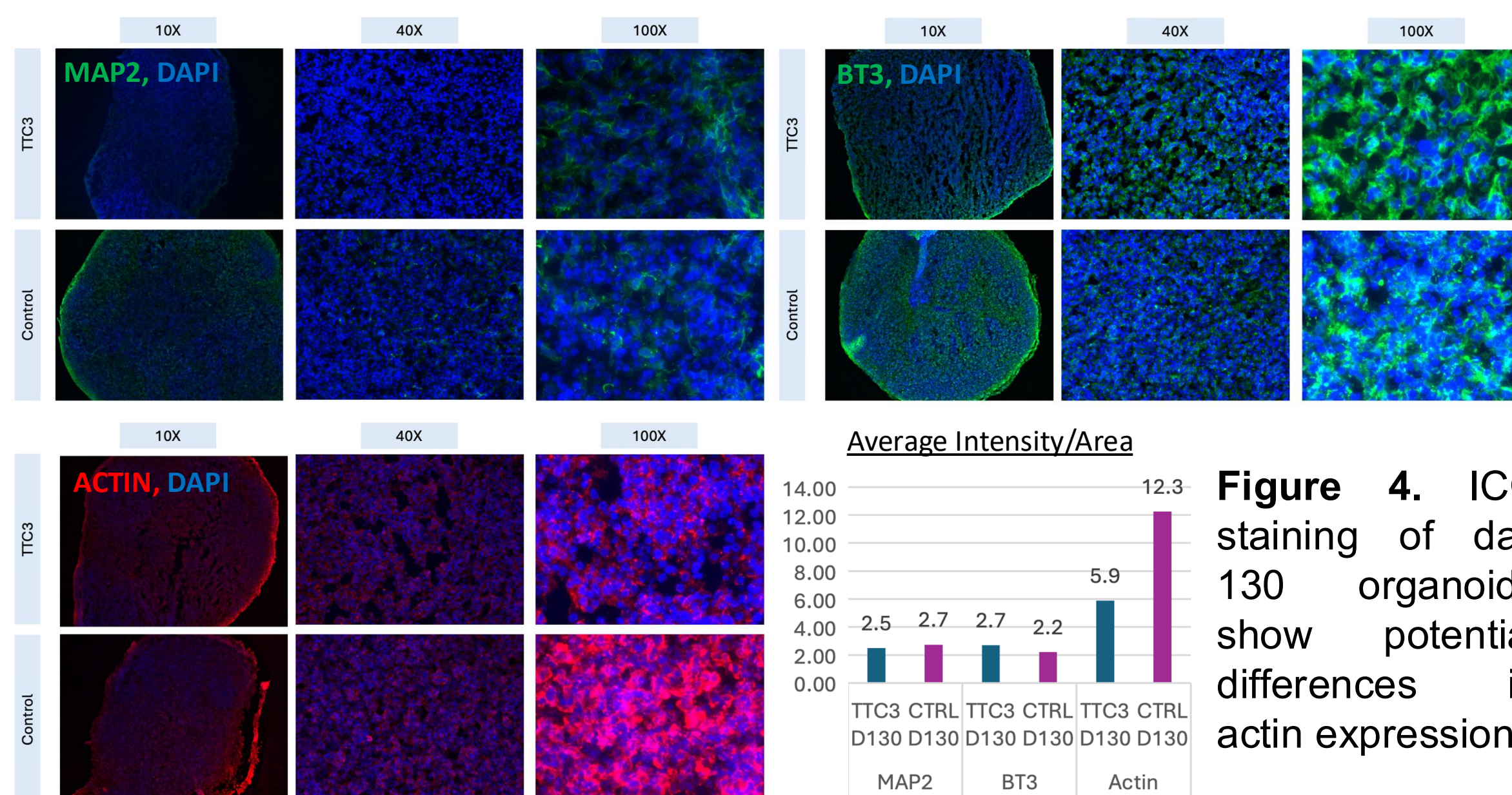


Figure 4. ICC staining of day 130 organoids show potential differences in actin expression.

METHODS AND RESULTS

The UMAP program was used to assign groups to distinct cell types and identify differentially expressed (DE) genes (**Fig. 5**). Quantitative evaluation of the cell type counts between the samples demonstrated there did not appear to be differentially expressed cell types between the control and *TTC3* samples.

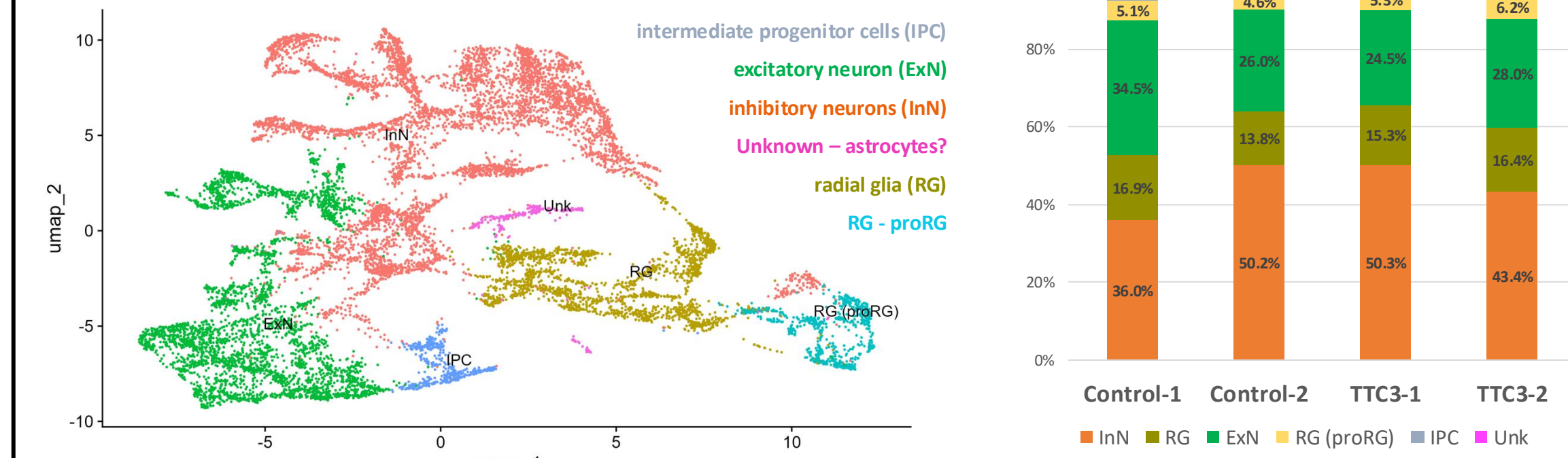


Figure 5. UMAP of all pooled organoid cells and quantification of cell types by each pool for both the unedited (control) and edited (*TTC3*) organoids.

TTC3 expression was significantly reduced in the edited line for four distinct cell types: excitatory neurons, inhibitory neurons, radial glia, and pro-radial glia, matching previous results in 2D neuronal cultures (**Fig. 2**). Some genes were significant altered across multiple cell types. For example, *carbonyl reductase 1* (*CBR1*) and *catechol-O-methyltransferase* (*COMT*) were significantly lower in the *TTC3* organoids in multiple cell types, and were the top two DE genes for radial glial cells. *CBR1* encodes for a NADPH-dependent oxidoreductase. Alzheimer mice (3xTg-AD) have increased levels of *CBR1* in their hippocampus. *COMT* assists in catalyzing neurotransmitters, such as dopamine, and has also been linked to Parkinson's disease. *COMT* has also been connected with reductions in both cognitive and motor function in aging individuals.

CONCLUSION

Utilizing CRISPR genome edited iPSCs in a 3D organoid model, we were able to identify potentially dysregulated genes by which *TTC3* may contribute to LOAD risk.

ACKNOWLEDGEMENTS

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