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Alcohol Use Disorder Associated Gene FNDC4 Affects Glutamatergic and GABAergic Neurogenesis in Human iPSC-derived Neural Organoids

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We previously performed a genome-wide association study (GWAS) for alcohol use disorder (AUD) drug treatment response (n=1,083) and that GWAS identified a genome-wide significant genetic locus which co-localized with a splicing quantitative trait locus (sQTL) for the fibronectin type III domain containing 4 (FNDC4) gene in multiple human brain regions. In addition, the FNDC4 sQTL in brain was also significantly associated with AUD risk in large-cohort GWAS. However, in spite of the fact that FNDC4 is highly expressed in the brain, its function remains unknown. Characterization of FNDC4 function in brain might help us understand molecular mechanisms underlying AUD pathophysiology and drug treatment response.

We investigated FNDC4 function using CRISPR/cas9 gene editing, the creation of human induced pluripotent stem cell (iPSC)-derived neural organoids and with single-nucleus RNA sequencing (snRNA-seq). Specifically, we generated FNDC4 homozygous knock-out (KO) iPSC lines and differentiated two single-colony KO iPSCs, together with wildtype (WT) iPSCs to generate forebrain organoids. Those neural organoids were harvested at three time points of differentiation (45, 90, and 150 days) for snRNA-seq. We found that KO of FNDC4 resulted in a significant and striking shift in the relative proportions of glutamatergic and GABAergic neurons in those iPSC-derived neural organoids, suggesting a crucial role of FNDC4 in neurogenesis. This series of observations suggests that FNDC4 may play a role in maintaining “balance” among neuronal cell types, which might contribute to the effects of alcohol on the brain as well as response to alcohol and AUD drug treatment.