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Identification of alcohol use disorder–associated and ethanol-responsive proteins in human brain organoids

Alcohol use disorder (AUD) is a chronic, relapsing condition with profound social, psychiatric, and medical consequences. As the functional products of genes, proteins provide more proximal insight into AUD mechanisms and potential therapeutic targets. However, protein abundance is highly tissue-specific and influenced by age, lifestyle, and comorbidities, making large, well-controlled studies difficult. Additionally, proteomic differences may reflect either inherited genetic liability or downstream effects of ethanol exposure.

To address these challenges, we generated human cortical brain organoids derived from induced pluripotent stem cell lines (iPSCs, 9 males, 7 females) and chronically intermittently treated them with 0 mM ethanol or a mean 24-hour ethanol concentration of 28.79 mM (0.13% BAC). We calculated polygenic scores (PGS) indexing genetic liability to AUD for each subject that we derived iPSC and organoid from, then innovatively incorporated PGS into our analyses to identify proteins associated with ethanol exposure (i.e., ethanol-responsive) or PGS (i.e., AUD-associated).

Quantitative proteomic profiling using data-independent acquisition identified 9,548 proteins from these organoids. Principal component analysis showed that the first principal component was associated with PGS ($P=0.0003$), whereas the second was associated with ethanol exposure ($P=0.04$), supporting distinct genetic and exposure-related proteomic signals. Using linear mixed-effect models with false discovery rate (FDR) <0.05, we identified 53 proteins associated with ethanol exposure; nine replicated in human postmortem brain tissues from 11 AUD cases and 16 controls. None of these proteins were associated with AUD, suggesting that they were ethanol-responsive only. While 1,806 proteins were nominally associated ($P<0.05$) with PGS, none survived FDR correction, underscoring the highly polygenic architecture of AUD. However, 24 of them are associated with AUD in GWAS catalog, indicating that they were AUD-associated but not ethanol-responsive. These preliminary findings suggest distinct genetic and ethanol-responsive proteomic signatures in AUD; more studies are needed to validate results and clarify underlying molecular mechanisms.

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