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# Epigenetic Regulation of GABA Catabolism in iPSC-derived Neurons: the Role of FGF21

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**Background:**
FGF21-based therapy is a potential treatment for substance use disorders i.e. alcohol or opioid misuse. FGF21 has a short half-life (0.5-2 hours) and crosses the blood-brain barrier. Our recent report showed plasma FGF21 levels positively correlated with alcohol use in patients with alcohol use disorder (AUD). Furthermore, ethanol could induce FGF21, which in turn altered catecholamine metabolism in induced pluripotent stem cell (iPSC)-derived brain organoids.

**Objectives:**
We set out to identify molecular mechanisms for both the naïve form of FGF21 and a long-acting FGF21 molecule (PF-05231023) in iPSC-derived neurons.

**Method:**
We performed RNA-seq and functional genomics studies i.e. co-immunoprecipitation, ELISA, and ChIP assay using iPSC-derived neurons (n=4) with and without drug exposure.

**Results:**
We identified 4701 and 1956 differentially expressed genes (DEG) in response to naïve FGF21 or PF-05231023, respectively (FDR<0.05). Notably, 974 DEG overlapped between treatment with naïve FGF21 and with PF-05231023. *REST* was the most important upstream regulator of DEG. GABAergic synapses were the most significant pathway identified using those overlapping genes. We also observed a significant positive correlation between plasma FGF21 and GABA concentrations in AUD patients (n=442). In parallel, FGF21 and PF-05231023 significantly induced GABA levels in iPSC-derived neurons. Finally, functional genomics studies showed a drug-dependent occupancy of REST, EZH2, H3K27me3 in the promoter regions of genes associated with GABA catabolism i.e.
*ABAT, GRBBR1*, and *SLC32A1,* which resulted in transcriptional repression.

**Conclusion:**
Our results highlight a significant role in the epigenetic regulation of genes involved in GABA catabolism related to FGF21 action.