Name: Diana Núñez-Ríos, PhD Email: diana.nunez@yale.edu

Glial Epigenomics of Opioid Use Disorder in the Human Postmortem Orbitofrontal Cortex

Background: Opioid use disorder (OUD) is associated with neuroadaptations in reward-processing circuits, including the orbitofrontal cortex (OFC). Epigenetic mechanisms—DNA methylation (5mC) and hydroxymethylation (5hmC)— are sensitive to environmental exposures and may modulate gene expression in OUD-affected brain cells. Our group previously identified OUD-associated 5mC and 5hmC alterations in neuronal cells isolated from the OFC. Given that glia express opioid receptors and opioid exposure can directly perturb glial homeostasis, we extended our investigation to OFC glial cells.
Methods: Glial cells were isolated by fluorescence-activated cell sorting from the OFC of 22 postmortem brains obtained from the VA Brain Bank. Cases (n = 9; OUD diagnosis; mean age of death 34.8 years; mean postmortem interval (PMI) 28.3 hours; 6 European, 3 African ancestry) and controls (n=13; non-OUD; mean age of death 39.3 years; mean PMI 29.6 hours; 10 European, 3 African ancestry) were profiled using 6-base sequencing, enabling simultaneous detection of 5mC, 5hmC, and genetic variation. Differential 5mC and 5hmC CpG sites and regions were identified with DSS and dmrseq R packages. Logistic regression models were adjusted for age at death, PMI, and the first three principal components (PC1–PC3). False Discovery Rate (FDR) was employed for multiple test correction.
Results: Single-site analysis identified two genome-wide significant CpG sites: one differentially methylated site (5mC; chr6:137406564) in an intergenic region, and one differentially hydroxymethylated site (5hmC; chr2:59078418) within LINC01122, a long intergenic non-coding RNA with high brain expression but as-yet uncharacterized downstream targets.
Discussion: This is the first study characterizing OUD-associated 5mC and 5hmC alterations in glial cells from the OFC. The limited epigenetic signal in glia - compared to the broader alterations we observed in OFC neurons – suggests that OUD-associated epigenetic dysregulation is more pronounced in neuronal than glial compartments of this brain region. Ongoing analyses will interrogate non-CpG methylation (CpH), to further elucidate cell-type-specific epigenetic mechanisms underlying OUD.

Authors:
Submitter: Diana Núñez-Ríos, Postdoctoral associate, Division of Human Genetics, Department of Psychiatry, Yale University School of Medicine, New Haven, New Haven, CT
José Martínez-Magaña, PhD, Division of Human Genetics, Department of Psychiatry, Yale University School of Medicine, New Haven
Janitza Montalvo-Ortiz, PhD, Division of Human Genetics, Department of Psychiatry, Yale University School of Medicine, New Haven
Melody Rivera-Hernández, PhD student, Division of Human Genetics, Department of Psychiatry, Yale University School of Medicine, New Haven

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