Regulatory signatures in primate neurons at cell type resolution

Alexey Kozlenkov [1, 2], Marit W Vermunt [3], Pasha Apontes [1], Junhao Li [4], Chet Sherwood [5], Yasmin L. Hurd [1, 2], Eran A. Mukamel [4,6], Menno P Creyghton [3], and Stella Dracheva [1, 2]

[1]James J. Peters VA Medical Center, Bronx, NY, USA; [2]Friedman Brain Institute and Departments of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [3]Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, the Netherlands; [4]Department of Cognitive Science, University of California San Diego, La Jolla, CA 92037, USA; [5]Department of Anthropology and Center for the Advanced Study of Human Paleobiology, The George Washington University, Washington, DC, USA; [6]Computational Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA

Recent GWASs for a variety of neuropsychiatric disorders provided evidence that common risk variants are typically located outside of coding regions of genes and are instead enriched within non-coding distal regulatory elements (mostly enhancers). Enhancers are known to display high level of tissue- and cell-type specificity. At the same time, brain is characterized by immense diversity of cell types, and only few epigenetic studies have been performed so far to probe the activity of enhancers in human postmortem brain in a cell-type-specific manner.

We have recently developed novel approaches to separate nuclei of excitatory glutamatergic (Glu) and inhibitory GABA neurons from the human autopsied prefrontal cortex (PFC). Using these methods, we have performed neuron-subtype-specific RNA-seq analysis as well as genome-wide profiling of several epigenetic marks, including DNA methylation/hydroxymethylation, and Histone 3 Lysine 27 acetyl (H3K27ac) modification. Because H3K27ac enrichment is a standard model for predicting position and activity of enhancers, these studies allowed us for the first time to map neuron-subtype-specific enhancers in the human brain. In addition, we compared H3K27ac marks in the Glu and GABA neurons across human, chimpanzee and macaque.

We identified neuron-subtype- and human-specific enhancers previously undetected in heterogeneous brain tissue, including human-specific regulatory changes in language-associated genes *FOXP2* and *CNTNAP2*. We observed preferential evolutionary divergence in neuron-subtype-specific enhancers, and a substantial proportion of positionally conserved enhancers undergoing subtype-specific alterations among the species. Finally, we assigned regulatory variation linked to psychiatric/neurological disorders to specific neuronal subtypes, and detected numerous human-specific enhancers near genes implicated in neurological and psychiatric diseases including drug addiction (i.e., *OPRM1).* Our study sheds new light on the interplay between regulatory evolution and the emergence of cell-type-specific gene expression programs, and refines predictions of genetic variants in disease, thus providing a valuable resource for further exploration of both neuropathology and normal brain function.