The largest repository of transcription factor binding data in human tissues compiled to date

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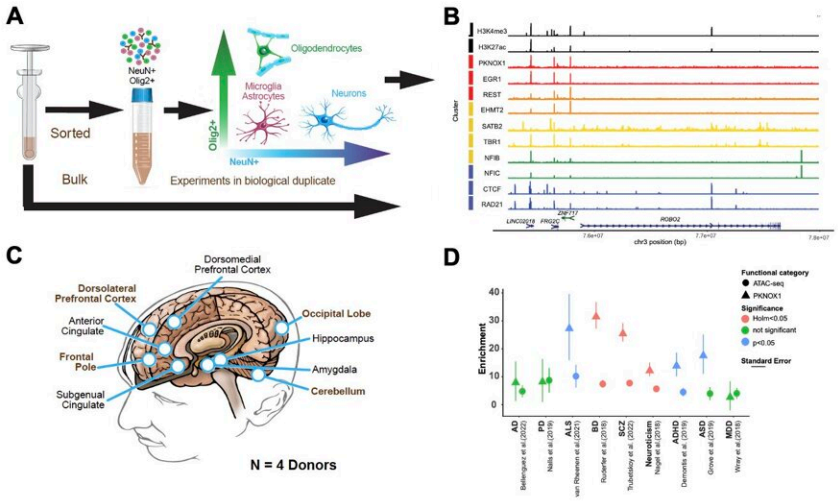

A) Process for isolating cell types from posthumous tissues for downstream experiments by breaking them up and labeling with antibodies. **B)** Example output of transcription factor binding experiments indicating the prevalence of each protein at locations throughout the genome. **C)** Overview of the brain regions in this study. **D)** Enrichment for psychiatric disorder risk variants in the binding sites of transcription factor PKNOX1.

Credit: Loupe et al. (*Nature Neuroscience*, 2024).

Transcription factors (TFs) are proteins that bind to specific DNA sequences, regulating the transcription of genetic information from DNA to messenger RNA (mRNA). These proteins play a pivotal role in the regulation of gene expression, which in turn impacts a wide range of

biological processes and brain functions.

While TFs have been the topic of numerous research studies, their binding dynamics in human tissue remain poorly understood. Shedding light on these binding dynamics could help to better understand how the expression of different genes contributes to the development of specific diseases, including neurodegenerative and psychiatric disorders.

Researchers at the HudsonAlpha Institute for Biotechnology, University of California-Irvine and University of Michigan recently carried out a study aimed at further exploring the contribution of TFs to gene expression and brain function. Their paper, published in *Nature Neuroscience*, presents the largest TF binding dataset compiled so far, thus opening new exciting opportunities for research.

"This project stems from my longstanding interest, since graduate school in the late 1970s, in understanding how genes are turned off and on in different types of cells, in disease and non-disease, in response to the environment (including drugs and exposures to different conditions) and during development and aging of an organism," Dr. Richard Myers, corresponding author for the paper, told Medical Xpress.

"My laboratory is particularly interested in the role of gene regulation in the human nervous system and the role that differential gene expression plays in neurodegenerative and neuropsychiatric diseases, as well as in normal brain function."

This recent study was carried out at the HudsonAlpha Institute by Dr. Jacob Loupe, Ashlyn Anderson and others in Dr. Myers's lab, as well as faculty investigator Dr. Greg Cooper. Its primary objective was to investigate the role of TFs, proteins that can recognize and bind to specific DNA segments, regulating the expression of nearby genes.

The DNA sequence included in a person's genome is identical across all the cells in his/her body. The crucial contribution of TFs is that they help to determine the form and function of these various types of cells.

"Much of the research in gene expression has been conducted in cultured cell lines that are grown in petri dishes in the laboratory," Jacob Loupe, Senior Researcher at HudsonAlpha, said.

"While this approach is invaluable to understanding gene regulation, there is great value in studying these systems directly in human tissues. By studying human cells from postmortem brain tissue, we seek to gain a better understanding of how gene regulation shapes healthy brain function and how disruptions may lead to psychiatric disorders such as schizophrenia and bipolar disorder."

To study TFs, the researchers used an innovative technique pioneered by Dr. Myers' lab in collaboration with Dr. Barbara Wold and her colleagues at Caltech. This technique is known as ChIP-seq, which stands for Chromatin ImmunoPrecipitation followed by Sequencing.

"Briefly, we use an antibody that is specific for each transcription factor to capture the protein from a sample and isolate the DNA fragments bound by the transcription factor," Loupe explained. "We then use instruments dedicated to reading ('sequencing') the millions of generated DNA fragments and use computational tools to match each sequence to its place in the genome."

As part of their recent study, Dr. Myers and his colleagues applied the ChIP-seq techniques to several dissected regions of the brain, focusing on almost 100 TFs. This allowed them to build a 'map' delineating brain regions in the genome that are activated or repressed by each TF.

"The experiments were carried out on nine different regions of the brain,

including the prefrontal cortex, which is associated with high-order cognitive functions, and the hippocampus, which is associated with memory," Loupe said.

"For some experiments, we utilized a technique known as flow cytometry to separate different cell types, such as neurons, so that we can get an even clearer picture of gene regulation.

"The postmortem tissues in this study were generously donated from four individuals and their families, without whom this research could not have been performed, through a long-standing collaboration with members of the Pritzker Neuropsychiatric Research Consortium at several universities around the U.S."

In addition to uncovering how individual proteins bind to human DNA, Dr. Myers and his colleagues carried out an integrative analysis, combining all the datasets they collected and identifying brain regions where protein-binding is plentiful, as well as regions where it is scarce.

They found that brain regions bound only by a few TFs may be the most interesting, as in these regions even a small change could have a significant impact on nearby genes.

"We have also incorporated other genome-wide studies that measure the association of genomic regions with particular diseases and found several transcription factors whose occupancies are significantly enriched for risk variants associated with neuropsychiatric disorders, predominantly in neurons," Myers said.

"Hopefully, our findings will help researchers in finding proteins and pathways that may serve as candidates for potential therapies."

The researchers carried out numerous experiments on multiple brain

regions belonging to different donors. This ultimately allowed them to construct what is, so far, the largest repository of TF binding data in human tissue.

The dataset they compiled could soon prove to be a valuable resource for scientists worldwide, allowing them to study TFs, gene regulation and their impact on specific brain functions. In addition, the dataset could help to unveil specific perturbations in TF binding that could contribute to specific diseases and psychiatric disorders.

"One of the fundamental challenges in genetics is understanding the link between gene regulation and disease," Myers said. "The ability of transcription factors to recognize a specific DNA sequence and affect nearby genes makes them indispensable in understanding this connection. One approach to understanding this mechanism is to look for variations in potential binding sites near genes of interest and infer their impact on downstream molecular pathways."

While probing variations in binding sites near genes of interest can lead to fruitful predictions, these predictions should be accompanied by experimental data. Specifically, neuroscientists should also measure interactions between TF binding and disease, to unveil the impact of changes to DNA sequences on their possible binding partners.

"We are currently performing a deeper analysis on the data generated in this study to investigate how the genetic differences between two donors can drive changes in transcription factor binding in health and in disease," Myers added.

"In addition, we have performed even more experiments in a larger, diverse cohort of donors with a history of various psychiatric disorders to see if there is an underlying signature of transcription factor activity associated with disease state and how specific changes to DNA may

drive that phenomenon."

More information: Jacob M. Loupe et al, Multiomic profiling of transcription factor binding and function in human brain, *Nature Neuroscience* (2024). DOI: 10.1038/s41593-024-01658-8