PERSPECTIVE

Mapping the brain's gene-regulatory maze

DNA sequences are connected to genes and functions in the developing and adult brain

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Genetic research has contributed greatly to the molecular diagnostics of neurodevelopmental and neuropsychiatric disorders. Nevertheless, the precise molecular causes underlying these disorders have often remained elusive, even in cases with clearly genetic causes. One of the reasons is the difficulty of linking genetic variants to genes and biological processes. On pages 862, 868, and 869 of this issue, Emani *et al.* (*1*), Deng *et al.* (*2*), and Wen *et al.* (*3*), respectively, as part of a collection of PsychENCODE2 papers, provide extensive molecular analysis of more than 1000 human brain samples of different ages and ethnicities. This dataset provides a broadly useful resource to map genetic variants to genes and biological functions, which benefits molecular diagnostics as well as basic research into the biology of the human brain.

Molecular assays, including microarrays and sequencing, have identified numerous genetic alterations that can have strong effects on brain development and on the risk of developing neuropsychiatric disorders (*4*–*7*). This includes large chromosomal deletions, recessive genetic variants, and dominant de novo mutations, which are typically rare within the population. Moreover, genome-wide association studies (GWASs) have pinpointed links between common genetic variants and the risk of developing certain disorders. These common variants tend to have weak effects; thus, GWASs require large cohorts to identify robust genetic associations (*8*).

Although these approaches are becoming better at detecting causative genetic variants, it can be difficult to connect an identified genetic variant or chromosome deletion to the gene(s) that contribute to the observed phenotype. Moreover, studies using whole-genome analysis have shown that some of the genetic risk for these disorders lies in noncoding sequences (*9*). However, interpreting these variants is notoriously difficult and compounded by context specificity, including the influences of tissue type, cell type, and developmental stage on gene regulation. This makes the task of identifying the impact of variants in gene regulatory sequences biologically complex and technically demanding.

To address these challenges, the PsychENCODE consortium was launched in 2015 as a multisite, multi-investigator effort aimed at generating multilayered data on gene regulation in the human brain. The current PsychENCODE2 collection provides a resource for identifying genomic regions that regulate gene expression in the brain across various developmental and adult stages. These studies analyzed gene expression and genotype of brain samples obtained from fetal tissue, healthy adults, and individuals with autism spectrum disorders, bipolar disorders, and schizophrenia (see the figure). Together, they provide a large resource that facilitates the identification of gene regulatory elements linked to the expression of genes across cell types and developmental stages of the human brain but also maps association with the expression of particular RNA transcripts such as isoforms and splicing variants. These studies highlight the complex, context-specific, and multilayered nature of gene regulation in the human brain, offering fresh perspectives on genetic susceptibilities to psychiatric disorders and providing a data hub for the systematic biological workup of genetic associations in brain disorders.

> **"...these three studies underline the importance of... biological context... of gene expression in the human brain."**

Focusing on the developing human forebrain, the largest region of the brain with central roles in higher cognitive functions and associated with a wide range of neuropsychiatric disorders, Deng *et al.* and Wen *et al.* characterized gene regulatory elements across different stages of fetal development. Wen *et al.* collected and analyzed gene expression profiles and the underlying genotypes for 672 brain samples, covering all gestational stages, to build a map of gene regulatory elements in the developing human forebrain. Their study connects genotypes not only with gene expression levels but also with gene isoforms and alternative splicing, which contribute substantially to the diversity of gene expression in the human brain.

Wen *et al.* also exploited the genetic diversity found in different ancestral backgrounds within their dataset to enhance the statistical power for precise genome mapping. By defining maps of genomic regulatory regions specific to different developmental stages, this resource facilitates future investigations of the developmental and genetic context in which neurodevelopmental disorders arise. The authors found that brain samples from the first gestational trimester were particularly enriched for cis-regulatory elements, highlighting the dynamism of gene regulation and the potential interplay between cell-intrinsic and context-specific effects.

Deng *et al.* took a complementary approach, identifying genomic regions that regulate gene expression, by applying massively parallel reporter assays (MPRA) in primary brain samples and organoids, combined with deep learning to uncover regulatory DNA sequences. The authors established a catalog of functional enhancers and computational models that predict cell type–specific regulatory regions. Deng *et al.* also addressed the effect of previously identified genetic variants on enhancer activity, suggesting that ~2% of the 8000 tested variants alter gene expression. Although this is a relatively small proportion, the presented analysis suggests that some of these genetic variants affect the expression of genes previously implicated in neuropsychiatric disorders. For example, the authors highlighted a genetic variant that potentially alters the expression of T-box brain transcription factor 1 (*TBR1*), a gene associated with autism spectrum disorder. Although further functional validation is necessary, this approach may be used to prioritize variants in the regulatory regions of disease-risk genes, potentially including not only causal variants but also protective alleles.

Complementing the developmental focus of Wen *et al.* and Deng *et al.*, Emani *et al.* established a large resource of genetic as-

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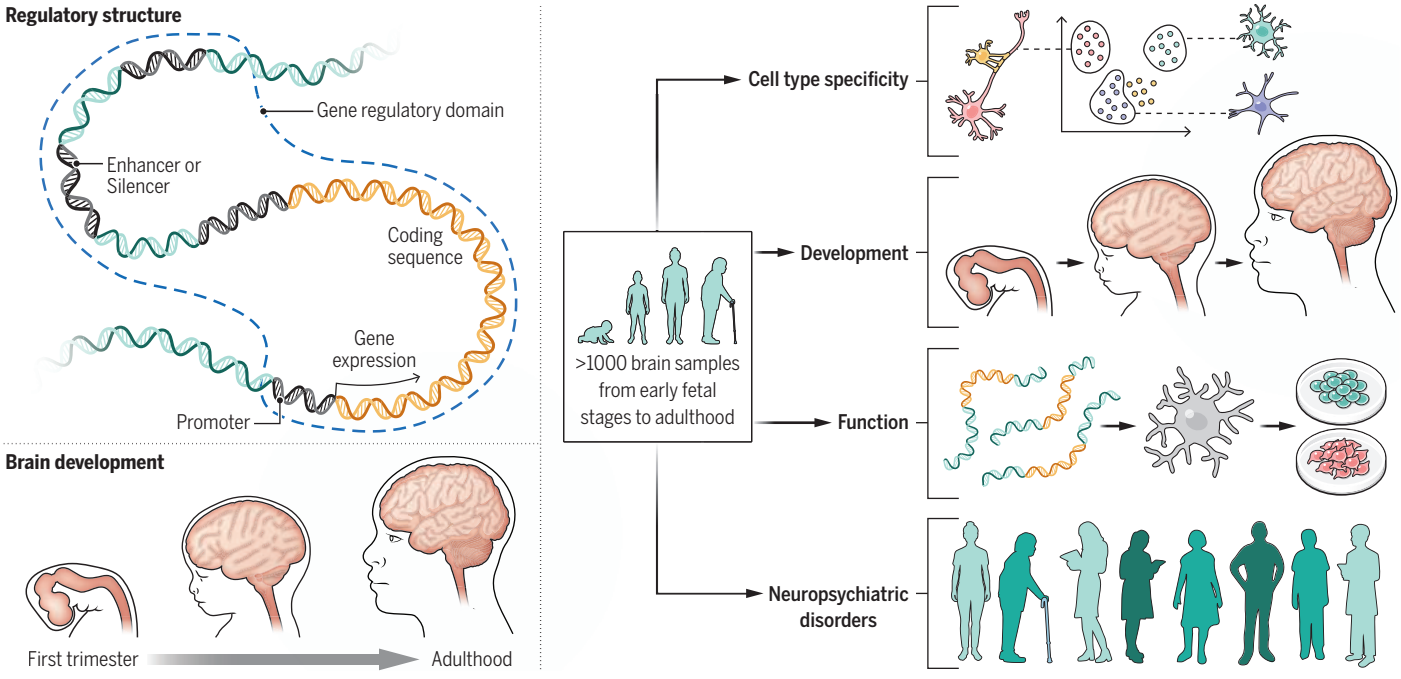
Exploring gene regulatory regions in the human brain

The PsychENCODE2 consortium uses a large cohort of human brain samples to examine functional regulatory elements across various cell types and developmental stages. This research offers a valuable resource for linking genetic variants to specific functions, including in neuropsychiatric disorders such as autism spectrum disorder, bipolar disorder, and schizophrenia.



sociations with gene expression in the adult prefrontal cortex, a brain region involved in complex cognitive tasks, decision-making, and social behavior. Addressing the challenge of identifying gene regulatory regions across the heterogeneous and complex landscape of brain cells, the authors employed single-cell RNA-sequencing and established brainSCOPE, a large single-cell genomics resource aimed at dissecting the functional genomic landscape of adult brains. This study exploits single-cell profiling in unraveling the cellular heterogeneity of the brain, offering a granular view of gene expression and regulatory elements across different cell types. This level of detail is crucial for understanding how genetic variations affect specific cellular pathways and thereby contribute to the phenotypes of neural disorders.

Recent years have seen a surge of research aimed at identifying therapeutic strategies to correct disease-causing mutations—for example, with CRISPR-based gene editing. At the same time, efforts are underway to develop drugs that target biological functions in specific cell types of the brain.

These studies mark a substantial step forward for neurogenetics by providing an overview of gene regulatory regions and associated gene expression patterns in the human brain. Such large-scale datasets and analyses help connect the human genome to the biology of the human brain, which is crucial for interpreting genetic data related to neurodevelopmental and neuropsychiatric disorders. Understanding these functions helps clarify how individual genetic variants contribute to the onset and progression of these disorders. Thus, these analyses offer important resources for interpreting genome sequencing data, allowing higher-confidence decisions about which genetic alterations are clinically relevant or constitute worthwhile starting points for drug discovery.

Given the complexities of chromatin structure and genome organization, a major challenge lies in linking gene regulatory regions to their target genes. There is thus great interest in new methods that can accurately map the connections between enhancers and genes within the intricate cellular mosaic of the brain. The combination of computational methods, developmental biology, and single-cell profiling can help unravel the genetic tapestry of the developing and adult human brain. The data provided in these studies constitutes an invitation for researchers to engage in new ways to analyze, integrate, and model these rich datasets.

Beyond the focus on gene expression, it would be interesting to profile multiple dimensions of regulatory cell states in the same samples, including epigenetic marks and chromatin profiles, protein levels, metabolic states, and spatial context. At the same time, the current studies already provide extensive material for functional studies to understand how the identified gene regulatory regions and their genetic variants affect brain-relevant traits.

Together, these three studies underline the importance of considering the influence of biological context, including cell type and developmental stage, on the genetic regulation of gene expression in the human brain. Thus, these findings highlight the need for a developmental lens when interpreting genetic data of neural disorders, suggesting that insights from adult brain studies may not fully capture the genetic underpinnings of disorders that originate in utero. Last, the ability to link genetic variants to specific cell types and context-specific regulatory roles creates new opportunities for molecularly targeted therapies, moving us closer to the realm of precision medicine in psychiatry. ■

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