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Regulatory landscape in brain development and disease

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Although many regulatory elements in the non-coding genome are linked to brain development and disease, deciphering their function has been challenging due to the lack of a genomic toolbox. However, recent advances in high throughput sequencing techniques have allowed us to begin decoding its function, enhancing our understanding of the regulatory landscape that underpins human traits and brain disorders. Here, we review how the regulatory landscape of the human brain undergoes dynamic changes across neurodevelopment, different cell types, and human evolution. We then discuss how regulatory landscapes shed light onto the molecular basis of neuropsychiatric disorders and guide the development of specifically targeted molecular therapies. Finally, we offer some thoughts on how these discoveries might impact the direction of future studies.

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Introduction

The dynamic nature of the genome across human brain development provides a unique avenue to study its implication in brain function and disease. The geneticist's toolbox has been rapidly expanding, allowing researchers to examine the regulatory landscape of the brain, its developmental dynamics, cellular complexities, and how its dysfunction contributes to disease. For example, RNA-seq has successfully delineated transcriptomic complexities of the brain across brain regions and development [1]. Regulatory codes and elements have been identified through various genomic resources including transcription factor binding sites (TFBS), quantitative trait loci (QTLs), ChIP-seq, and ATAC-seq. Hi-C has greatly advanced our understanding of how chromatin is folded in the nucleus, highlighting the importance of distal chromatin interactions in gene regulation [2,3]. Single-cell sequencing technologies are reengineering these tools to decipher regulatory dynamics in a cell-type specific manner [4]. In combination, these tools allow us to decipher how genetic sequences drive gene regulation via genetic variation (QTLs), trans-regulators (ChIP-seq), chromatin accessibility (ATAC-seq), and chromosome conformation (Hi-C) in the human brain (Table 1).

Here we summarize our current knowledge of the regulatory landscape of the human brain across development and different brain cell types. We review how functional genomic datasets can help decipher the regulatory logic of the human brain and highlight the need to study psychiatric disorders in a temporal-specific and cell type-specific fashion (Figure 1). Finally, we discuss the implication of human evolution in shaping the regulatory architecture of brain development and function.

Regulatory landscape of the developing brain shapes neuropsychiatric disorder risk

Transcriptomic profiling across the developmental time span indicated highly dynamic transcriptomic landscape with a sharp transition between prenatal and postnatal stages [5•]. Prenatal to postnatal transition in gene expression coincides with changes in chromatin architecture that encompass enhancers (measured by H3K27ac) and chromatin interaction (measured by Hi-C) [5•,6]. Chromatin interaction and accessibility profiles in two layers of the developing cortex, the cortical plate (CP, enriched for postmitotic neurons) and germinal zone (GZ, enriched for neural progenitors), further demonstrated dynamic chromatin architecture during key processes during neurodevelopment such as cortical neurogenesis and neural differentiation [9,12]. In line with these findings, co-expression networks built across human brain development showed that transcriptional regulators and chromatin remodelers peak during neurodevelopment, suggesting extensive chromatin rewiring that accompanies cell type composition changes [14]. Based on a recent study showing that the combination of enhancer activity and chromatin contact frequency accurately predicts gene regulatory architecture [15], chromatin interaction and accessibility profiles across development would provide important insights into the gene regulatory mechanism that is critical for understanding proper brain development and function.

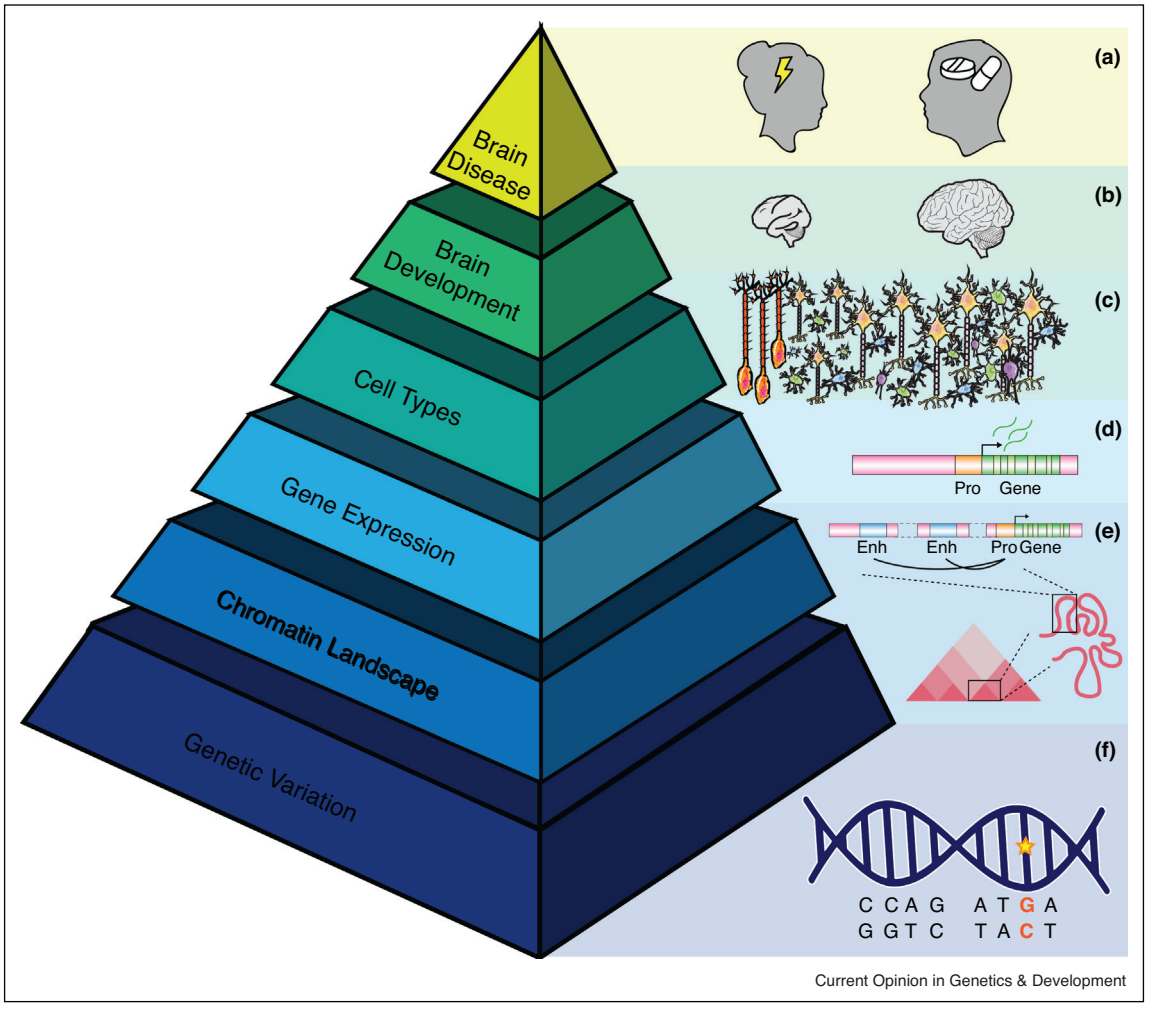
Another important *cis*-regulatory element includes eQTL: genetic variation associated with gene expression

Table 1

Chromatin architecture in the human brain tissue

Technique	Measures	Available datasets
ChIP-seq	Histone modification and transcription factor (TF) binding sites	Fetal brain: [5•] Adult brain: [6] Sorted neurons and glia: [7•,8]
ATAC-seq	Chromatin accessibility	Fetal brain: [9] Adult brain: [10] Sorted neurons and glia: [11]
Hi-C	Three-dimensional chromatin interaction	Fetal brain: [12] Adult brain: [6,13] Sorted neurons and glia: [7•,70]

Figure 1



The regulatory landscape of the brain serves as a basis for understanding the molecular, cellular, and developmental underpinnings of brain disease. Genetic variation **(f)** can lead to changes in chromatin architecture **(e)**, which in turn affect gene regulation. Gene expression **(d)** changes in one or multiple cell types **(c)** can perturb brain development **(b)**. Alterations in any of these layers contributing to brain function throughout the lifespan of an individual can result in a variety of brain diseases **(a)**. Enh, enhancer; Pro, promoter.

(Box 1). So far, three studies have reported eQTLs identified in the fetal brain [16–18]. While some fetal brain eQTLs continue to be associated with gene expression into adulthood, many fetal brain eQTLs linked to neuropsychiatric disorder risk loci are temporal-specific [16–18]. This temporal-specific pattern could be due to the cellular composition change during neurodevelopment, which requires further investigation. These results

Box 1 Building blocks and emerging principles of gene regulation

The epigenetic landscape acts as a blueprint for gene regulation throughout the lifespan of an organism. Here, we briefly discuss a few building blocks of the *cis*-regulatory landscape (regulatory architecture that affects nearby regions within a chromosome) that have been used to infer cognate genes for non-coding risk variants, and how they can be studied.

TADs: Topologically associating domains (TADs) define frequently self-interacting regions of the genome [33–35]. TADs often define the boundaries for enhancer-promoter interactions, and hence play a critical role in gene regulation. Disruption of TAD boundaries is coupled with abnormal enhancer rewiring, and subsequent gene dysregulation is associated with a wide range of diseases including cancer and congenital limb deficiencies [36–39]. A recent study has also shown that the expansion of tandem repeats in fragile X syndrome leads to the disruption of TAD boundaries, suggesting a role of TADs in brain disease [40].

Chromatin interactions: While TADs define the boundaries of grouped enhancers and promoters, the fine-tuning of gene regulation is mediated by chromatin interactions within TADs. Enhancer-promoter interactions, one of the most representative examples of chromatin interactions, have been shown to play a pivotal role in gene regulation and have been widely used to link non-coding variation to putative target genes [6,12,13]. However, there are other types of chromatin interactions (e.g. promoter-promoter interactions) whose role in gene regulation remains elusive, necessitating additional studies to unveil currently unknown function of chromatin interactions in gene regulation.

ABC model: Chromatin interactions are defined based on a statistical model that measures significance of interactions compared to the local neighborhood or expected distance-matched contact frequency. A recently proposed activity-by-contact (ABC) model complements chromatin interactions by taking actual chromatin contact frequency into account. It employs an equation that multiplies the enhancer activity and its contact frequency with the gene promoter to predict the contribution of an enhancer to gene expression [15].

CRD: Previous studies have shown that co-accessibility between two regulatory elements can be used to construct cis-regulatory networks and infer putative target genes of regulatory elements [9,41]. Cis-regulatory domains (CRDs) further elaborate this strategy by defining domains based on interindividual correlation between chromatin activity peaks [42]. CRDs resemble TADs in that they regulate gene expression by bringing together different regulatory elements such as enhancers and promoters, but are finer in scale and highly dynamic. Importantly, CRDs provide insights into how non-coding variation impacts gene regulation by linking active regulatory elements to gene expression.

eQTL: A large pool of genetic variation and gene expression profiles from hundreds of individuals allows us to identify genetic variation that is statistically correlated with gene expression. Termed expression quantitative trait loci (eQTLs), these regulatory variants are playing an increasing role in inferring the target genes and predicting the biological impact (quantitative changes in gene expression) of the non-coding risk variants.

Notably, different approaches to capture regulatory dynamics converge to reveal similar regulatory networks and reinforce findings across studies and techniques. For example, chromatin activity-based CRDs share similar domain-like structures with chromatin contact-based TADs [42]. On a finer scale, about 30% of eQTLs are also supported by Hi-C-based chromatin interactions [6]. By combining multiple measures, we are able to enhance the predictive power of gene regulatory networks, as shown by the accuracy of the ABC model [15]. Therefore, amalgamation of multi-faceted functional genomic datasets that include physical proximity, regulatory elements, and regulatory variants would provide mechanistic insights into how genes are regulated.

highlight the importance of deciphering developmental stage-specific regulatory codes in order to understand the impact of genetic variation on transcriptional regulation across brain development [16–18].

Because of the unique chromatin architecture and *cis*-regulatory variation during brain development, many studies have been conducted to assess their role in the development of psychiatric disorders. Intriguingly, genes that harbor rare *de novo* mutations in autism spectrum disorder (ASD) were enriched for transcriptional regulators expressed during early brain development, suggesting that their dysregulation in the developing brain may lead to neurodevelopmental disorders [14,19,20]. In addition, genes with rare *de novo* mutations in schizophrenia formed co-expression and protein interaction networks in the developing prefrontal cortex (PFC) [21].

Similar findings have been observed for common variation associated with psychiatric disorders. Cross-disorder meta genome-wide association studies (GWAS) have found genomic regions associated with the development of multiple neuropsychiatric disorders (pleiotropic loci) [22,23•]. Putative target genes of the pleiotropic loci showed elevated expression during fetal brain development, suggesting that pleiotropy among psychiatric disorders may have shared neurodevelopmental origins. Genes associated with individual psychiatric disorders also showed similar enrichment during fetal brain development [24•].

Despite the importance of decoding neurodevelopment in psychiatric disorders, the majority of epigenetic and transcriptomic profiling has been conducted in adult postmortem brain tissue because brain development precedes diagnosis [25,26]. Human brain organoids can provide an attractive model system to fill this gap by recapitulating cellular diversity [27] and epigenetic programming [28•] of the developing human cortex. In addition, organoids can model early corticogenesis, which is a developmental stage from which *in vivo* tissue is difficult to obtain [29]. On the contrary, another study reported that organoids do not fully recapitulate cellular subclasses, areal specification, and maturation observed during *in vivo* brain development [30], highlighting the need for optimization and standardization of this miniature brain-in-a-dish to unveil neurodevelopmental disease mechanisms. Collectively, epigenetic and transcriptomic characterizations of organoids that are derived from patients with high polygenic risk scores or

loss-of-function mutations in neurodevelopmental disorder risk genes [19,20,31] could permit the identification of a disease mechanism operating during the critical period for causing psychiatric disorders.

Given that the earliest traces of many psychiatric disorder risk genes converge on neurodevelopment, targeted treatments may need to be designed to act during this stage. In line with this finding, hyperexpansion of the cortical surface area has been found in asymptomatic infants later diagnosed with ASD [32], suggesting that the developmental alterations may arise earlier than the behavioral symptoms. An early intervention that prevents the improper downstream cascades before behavioral symptoms develop may be useful in treating psychiatric disorders.

Cell-type specific gene regulatory landscape and its implications for psychiatric disorders

The human brain is exquisitely complex in its cell-type composition. In order to achieve such cellular diversity, different cell types emerge and disappear across different developmental time periods, suggesting that cellular complexity partly underscores highly dynamic developmental transcriptomic landscape [5•].

Recent development of single-cell sequencing technology has enabled deciphering cell-type specific gene regulatory architecture. One of the approaches, single cell RNA sequencing (scRNA-seq), has elucidated the diversity in transcriptomic signatures among brain cell types and has shed light on groups of genes that mark distinct cell types [43–45]. Notably, gene expression signatures distinguished neuronal subtypes previously identified based on morphology and spatial organization, as well as previously unknown neuronal subtypes [44]. Further, cellular expression profiles can be used to construct lineage-specific trajectories of cellular differentiation and maturation in the developing cortex [43].

Given the diverse functions of different cell types in the brain, it is not surprising that different cell types exhibit different regulatory architecture to support their specific functions. For example, expansion of glia and subsequent cellular heterogeneity in the adult cortex is a major factor that differentiates chromatin architecture of the adult cortex from the developing cortex [6]. Likewise, epigenetic profiling of iPSC-derived neurons and astrocytes as well as sorted cells from the adult cortex have demonstrated cell-type specific regulatory landscapes that include enhancers and three-dimensional chromatin interaction [7•,8,46•,70]. Furthermore, the process of neural differentiation is accompanied by dynamic chromatin rewiring that involves changes in compartment, TAD, and enhancer-promoter interactions [12,46•,47]. Therefore, it is critical to study chromatin dynamics in a cell-type specific fashion in order to capture the full range of diversity and complexity of chromatin architecture and consequential gene regulation. However, single cell epigenetic datasets (e.g. scATAC-seq or scHi-C) of the human brain are limited compared with scRNA-seq. Recent development of joint single cell profiling assays [48] as well as computational frameworks that integrate multiple single cell genomic datasets [49] will help decode cell-type specific gene regulatory landscape that underlies cellular expression signatures.

Remarkable transcriptomic diversity among different brain cell types suggests that cellular expression signatures can be used to identify central cell types associated with disease. Several tools have been developed to systematically identify cell types associated with GWAS traits [24•,50–52]. These studies found that genes associated with psychiatric disorders including schizophrenia and ASD were highly expressed in excitatory neurons [6,8,24•,46•,50,51,53], while genes associated with neurological disorders were highly expressed in glia [24•,54,55]. Together, these studies highlight the importance of understanding the contribution of different cell types to the development of neuropsychiatric disorders, as different cell types may have unique roles in disease development [51].

Additionally, genetic variants identified as risk loci in GWAS for different brain disorders may have distinct regulatory roles in different cell types due to the cell-type specific chromatin architecture [7•,46•]. For example, Alzheimer's disease associated SNPs physically interact with *BIN1* in a microglia-specific manner, and the deletion of the SNP-containing enhancer leads to down-regulation of *BIN1* only in microglia [7•]. Similarly, schizophrenia-associated SNPs physically interact with *PCHD* genes in iPSC-derived neurons and neural progenitors, but not in astrocytes [46•]. Given that psychiatric disorders are strongly associated with excitatory neurons [24•], transcriptomic and epigenomic characterization of neuronal subtypes will be critical to deciphering the precise biological impact of psychiatric disorder risk loci. Finally, cell-type specific eQTL resources would reveal how genetic variation is associated with cellular expression profiles.

Unique human gene regulatory circuits for unique human traits

Emergence of complex human behaviors mirror changes in our brain structure that involve cortical expansion and elaboration [56]. Given the high similarity of protein-coding sequences between human and non-human primates, the evolution of regulatory elements and resulting changes in gene expression has been proposed to play a major role in human evolution [57]. Indeed, a recent comparison of transcriptomic signatures between human and rhesus macaque across cortical development has revealed that protracted maturation is a unique feature

of the human cortex [58]. Regulatory elements including human accelerated regions (HARs) and human-gained enhancers (HGEs) may contribute to this human-specific protracted cortex maturation [59]. HARs involve human-specific sequence changes in the genome [60], while HGEs involve changes in enhancer usage [61,62]. Putative target genes of HARs and HGEs based on Hi-C evidence suggest that these elements are highly expressed in radial glia, a cell type associated with enhanced neurogenic niche and cortical expansion [9,12,59]. Recent CRISPR-based genetic screening has further confirmed their role in neurogenesis [63]. Intriguingly, both HARs and HGEs interact with neurodevelopmental disease risk genes, implicating the contribution of human brain evolution to disease susceptibility [59]. Independent findings have also reported that copy number variation (CNV) and biallelic mutations impacting HARs are enriched in individuals with ASD [64]. In addition, genes associated with neuropsychiatric disorders were tightly linked to the expression profile of radial glia, highlighting their role in cortical evolution and psychiatric disorders [22,24•]. These results collectively suggest that human evolutionary regulatory elements are associated with psychiatric disorders, underscoring the fact that many of these disorders are unique to human behaviors and traits.

Taken together, evolution of gene regulatory circuits leads to gene expression changes that are unique to humans. Human-specific gene regulation may have different effects on different cell types, especially on radial glia and progenitor cells which contribute to human cortical evolution and expansion [65]. Given the role of radial glia in determining the number and type of brain cells during corticogenesis, changes in their gene expression profiles may have a profound impact on brain development and function. Therefore, it is imperative to consider human-specific regulatory elements and their impact on neurodevelopment to better understand the complexity behind human traits and behavior.

Conclusion

The complexity of the human brain originates from its complex developmental trajectories, unparalleled diversity of cellular composition, and intricate neuronal wiring within brain circuits. Recent advances in genomic technologies have begun to discern the gene regulatory principle that governs developmental dynamics, cellular diversities, and brain circuits. Distinct transcriptomic signatures have been observed in different brain regions at different stages of development, indicating that divergent regulatory strategies are used for different brain regions across development [5•]. This developmental and regional transcriptomic diversity is tightly coupled with cellular complexity, as different classes of cells are detected in different stages and brain regions. It is increasingly recognized that different cell types exhibit distinct gene regulatory landscapes, which would affect transcriptomic signatures and morphological features [7•,46•]. However, we are far from identifying a comprehensive regulatory principle. First, the majority of studies are conducted in the cortex, while subcortical regions also play an important role in brain function and disease. Second, cells do not work in isolation, highlighting the need for spatial mapping, which provides connectivity within brain regions, layers, and microcircuits. Spatial mapping of cellular expression and epigenetic signatures may help reconstruct the brain circuits at a single cell resolution [66]. Third, the developmental dynamics of cell-type specific transcriptomic and epigenetic signatures are not well understood. Single cell lineage tracing would be able to delineate the full spectrum of cellular diversity across developmental trajectories [67]. Fourth, the electrophysiological property of a cell is a key feature for fully capturing the functional diversity across different neuronal subclasses, while its relationship to molecular signature needs to be further examined [68,69]. Finally, neurons have a unique feature of activity-dependent transcription, and the precise regulatory logic underlying this process is yet to be identified [71].

Importantly, brain disease needs to be understood as a whole. The functional impact of a risk variant needs to be carefully examined in a developmental stage, cell-type, and brain region specific manner. Understanding the gene regulatory principle that spans multiple brain regions, developmental stages, and cell types is therefore essential to providing mechanistic insight into psychiatric disorders.

Conflict of interest statement

Nothing declared.

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