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The Developmental Transcriptome of the Human Brain: Implications for Neurodevelopmental Disorders

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Abstract

Purpose of review—Recent characterizations of the transcriptome of the developing human brain by several groups have generated comprehensive datasets on coding and noncoding RNAs that will be instrumental for illuminating the underlying biology of complex neurodevelopmental disorders. This review summarizes recent studies successfully utilizing these data to increase our understanding of the molecular mechanisms of pathogenesis.

Recent findings—Several approaches have successfully integrated developmental transcriptome data with gene discovery to generate testable hypotheses about when and where in the developing human brain disease-associated genes converge. Specifically, these include the projection neurons in the prefrontal and primary motor-somatosensory cortex during mid-fetal development in autism spectrum disorder and the frontal cortex during fetal development in schizophrenia.

Summary—Developmental transcriptome data is a key to interpreting disease-associated mutations and transcriptional changes. Novel approaches integrating the spatial and temporal dimensions of these data have increased our understanding of when and where pathology occurs. Refinement of spatial and temporal properties and expanding these findings to other neurodevelopmental disorders will provide critical insights for understanding disease biology.

Keywords

psychiatric and neurologic disorders; genetics; gene co-expression analysis; autism spectrum disorder; schizophrenia; Williams syndrome

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Introduction

The human brain is an immensely complex biological system, composed of many functionally distinct regions, neural circuits and cell types. Furthermore, its development is a highly complex and strictly regulated process that transpires over an extended period of time, during which changes occur at both the structural and functional levels. This process is exquisitely dependent on the appropriate expression of intact gene products (RNA and protein). Mutations that alter gene expression or function can result in or contribute to neurological and psychiatric conditions ranging from intellectual disability to autism spectrum disorder (ASD) and schizophrenia (SCZ).

Recent advances in high-throughput and genome-wide analyses of the transcriptome using human post-mortem tissue have shown that the intricate features of human brain development and organization are reflected in the organization and complexity of its transcriptional architecture (1–9). In particular, the initial comprehensive analyses of the human brain transcriptome have revealed the remarkable dynamism of coding and non-coding transcripts during prenatal and early postnatal development of the human brain (1, 3, 5) (www.brainspan.org). Furthermore, they have provided critical insights into the trajectories of genes associated with specific neurodevelopmental processes (Figure 1). These studies have demonstrated correlations between gene expression dynamics and the morphological and functional specialization of brain regions, and consequently, provide an avenue to dissect the specific molecular underpinnings of this specialization, and importantly, how it may go awry in neurodevelopmental disorders. Thus, research into the molecular aspects of normal human brain development using post-mortem human tissue has created a critical resource for understanding the etiology of a range of complex and poorly understood disorders.

In this review, we interpret recent studies of differential gene expression in patients with psychiatric and neurological disorders against the backdrop of these comprehensive developmental human brain transcriptomes. We then highlight recent studies in which these transcriptome data are integrated with genome-wide gene discovery efforts in a spatially and temporally informed analysis to gain deeper insight into manifestations of these disorders.

Autism spectrum disorders

With regard to protein-coding genes and complex neurodevelopmental disease, genes linked to both syndromic and idiopathic forms of autism spectrum disorders (ASDs) show varied developmental expression trajectories (10–16). For several of the earliest identified genes associated with idiopathic ASD, such as *neurexin 1 (NRXN1)* and *neuroligin 4, X-linked (NLGN4X)*, their expression increases during late prenatal development and peaks just after birth in the cerebral cortex, as would be predicted given their function in synapse development (9). Additional insight were gained by performing gene ontology (GO) enrichment analysis on the group of genes co-expressed with *NRXN1*, *NLGN4X*, and other ASD-related genes expressed during normal human brain development. This analysis assesses the GO terms (biological descriptors) for each gene in the group and determines if there is statistically significant recurrence of GO terms among the genes in the group; with

enrichment indicating shared biology between the genes. Here, GO enrichment analysis identified enrichment of categories such as ‘phosphoprotein,’ ‘synapse,’ and ‘neuron projection’ (5). However, for other genes associated with idiopathic forms of ASD, their expression patterns and cellular localizations are less informative with respect to their role(s) in normal or abnormal brain development.

One strategy for addressing the lack of immediately obvious mechanistic insights emerging from gene discovery efforts is to analyze global changes in gene expression, comparing primary affected versus unaffected brain tissue. The first transcriptome-wide effort in this regard in autism evaluated transcriptional differences between normal and ASD frontal neocortex (NCX), temporal NCX, and cerebellum, and used weighted gene co-expression network analysis (WGCNA) to identify highly related changes in gene expression (17). Though these ASD samples did not have a known genetic risk in common, the authors were nonetheless able to find convergence of expression changes corresponding to GO terms such as ‘synapse,’ ‘neurotransmitter transport,’ and ‘neuron projection.’ Genes within these categories included *RBF1* (also known as *A2BP1* or *FOX1*), an exon splicing factor previously implicated in ASD (18), and *CNTNAP1* (also known as *P190* or *CASPR*), an axonal transmembrane protein capable of multiple protein-protein interactions (19), both of which have very dynamic developmental expression patterns in the NCX. Another study utilizing postmortem tissue from brains of autistic individuals examined the dorsolateral prefrontal NCX and found dysregulation in genes that are known to regulate neurogenesis and neurodevelopment (20).

Studies of gene expression in the developing human brain have also recently led to surprising and provocative findings relevant to syndromic forms of autism, such as Fragile X. For example, a recent study interrogating layer- and region-specific expression of neuronal *nitric oxide synthase 1* (*NOS1*) in mid and late-fetal glutamatergic excitatory cortical projection neurons, also called pyramidal neurons, identified posttranscriptional regulation by the RNA-binding protein fragile X mental retardation protein (FMRP). Although *NOS1* mRNA is transiently widely expressed in fetal human and mouse NCX, only primate transcripts have gained a sequence motif for binding to FMRP that enables efficient translation in a subset of cortical projection neurons (21). *NOS1* protein levels are dramatically reduced in postmortem developing human Fragile X NCX, but not in mouse disease model (i.e., FMRP-deficient mouse). These findings suggest a novel etiological mechanism for intellectual disability and ASD via nitric oxide signaling and identify specific cell types and developing neural circuits affected in Fragile X syndrome (21). In addition, this study illustrates why it is important to study species-specific aspects of brain development and how this can lead to a better understanding of neural circuit dysfunctions in human cognitive disorders.

Viable alternatives to post-mortem human brain tissue analysis may be cell lines and induced pluripotent (iPS) cells derived from patients diagnosed with ASD (22). In one recent example, investigators isolated stem cells from exfoliated deciduous teeth of patients with idiopathic ASD and compared their transcriptomes to control cell lines (23). They found that roughly half of the differentially expressed genes were expressed in the brain, and of those, the functional ontologies most overrepresented were ‘neurogenesis’ and ‘neurite

outgrowth'. Highly up-regulated genes included those that function in alternative splicing, a process previously implicated in the etiology of ASD (24). However, it is still unclear if these results reflect the origin or consequence of brain developmental abnormalities in patients with ASD.

Copy number variations (CNVs) involving multiple genes have been found to carry large risks for ASDs in 5–10% of the affected population. CNVs frequently associated with ASDs include those mapping to 1q21.1, 7q11.23, 15q11.2, 15q11.2-13.1, 15q13.2-13.3, 16p11.2, and 22q11.21 (13, 25–31). Comparing lymphoblasts from probands with confirmed ASD-associated CNVs to lymphoblasts from matched unaffected siblings, researchers have investigated transcriptional differences and found that the genes that were misexpressed in ASD-affected individuals, but not unaffected siblings, function in neural-related pathways including neuropeptide signaling, synaptogenesis, and synaptic contact (32). Several convincing relationships between genes differentially expressed in individuals with specific CNVs and neurodevelopmental processes were observed, despite these experiments being carried out in lymphoblastoid cell lines. CNVs at 16p11.2 are associated with clinical micro- or macrocephaly, and these authors showed that the degree of misexpression of *TAOK2*, *CORO1A*, *KCTD13*, and *QPRT*, which have the largest expression changes dependent on the locus copy number, but are not within the locus itself, and are expressed during NCX development, account for more than 50% of head circumference variance (32). Similarly, duplications in the Williams syndrome locus 7q11.23, recently associated with idiopathic ASD (13) resulted in 85 differentially expressed genes that reside outside of the locus. These 85 genes fall into gene ontologies including 'forebrain development', 'bilateral symmetry', and 'hippocampus development.' These studies highlight the benefit of transcriptional profiling of patient cells, including those from non-neural tissues.

These studies, while informative, do not generate testable hypotheses about the specific time periods, brain regions, and/or cell types in which ASD may be productively modeled. However, efforts aimed at gaining this type of dimensionality have recently emerged, leveraging the multi-dimensional nature of gene expression datasets across the developing human brain. With respect to autism, several studies have combined ASD gene discovery with the BrainSpan developmental transcriptome to address these issues.

An initial assessment of the genes identified by whole-exome sequencing of simplex ASD families (10, 12, 14, 15) using the BrainSpan exon array transcriptome dataset (5) and WGCNA, highlighted the potential role in ASD pathobiology of chromatin regulation during early brain development (33). However, specific brain regions and time periods of development were not demarcated in this study.

In a more recent study however, Willsey *et al.* leveraged the same spatio-temporal map of gene expression (5). They focused on nine genes most strongly associated with ASD based on recurrent *de novo* loss of function mutations and set out to determine when and where in the developing and adult human brain, as well as in which cell types, co-expression networks defined by these ASD-genes, converged (34). Importantly, these ASD genes were identified by exome- and genome-wide sequencing methods and not biased by mechanistic hypotheses or prior notions of biological plausibility. Using a 'bottom-up approach' and

building around the nine high confidence ASD ‘seed’ genes, the authors created co-expression networks by brain region and by developmental period and assessed them for enrichment of other ASD risk genes identified by whole-exome sequencing. Strikingly, enrichment of ASD risk genes was localized to two developmental epochs and brain regions only, with the strongest signal of convergence observed in the prefrontal and primary motor-somatosensory cortical regions during midfetal development (10–24 post-conceptual weeks). Incorporation of an additional gene expression dataset comprised of laser microdissected NCX layers from embryonic human brain as well as known cell-type specific marker genes further implicated cortical projection neurons in NCX layers 5 and 6 (Figure 2A).

In an alternative “top down approach” utilizing the RNA-seq version of the BrainSpan developmental transcriptome (www.brainspan.org) and focusing on fetal and early postnatal brain development in the human NCX, Parikshak *et al.* (36) employed WGCNA to build modules of co-expressed genes and identified relevant modules by mapping lists of ASD-associated genes. Downstream analyses subsequently assessed shared neurobiological function among the genes within these modules and also localized modules to specific layers and cell types. In addition to implicating deep layer cortical projection neurons, as in the study by Willsey *et al.*, this study also highlighted superficial cortical projection neurons. Together these studies underscore the utility of integrating a spatially and temporally rich gene expression data from a neurodevelopmental context and strongly associate mid-fetal brain development, particularly of cortical projection neurons of the frontal cortex – suggesting that future mechanistic studies should involve these cell types.

Williams syndrome

Duplications at the locus 7q11.23 have been associated with ASD (13) as well as schizophrenia (37), while deletions of the identical interval result in Williams syndrome (WS; also Williams–Beuren syndrome). Interestingly, individuals with WS demonstrate a remarkably consistent behavioral phenotype characterized by a high degree of social interest and affiliativeness. This presents a striking contrast to the core features of ASD. While comparatively fewer studies have examined transcriptional changes from WS tissue, two recent studies suggest how these changes may affect brain development in WS. Using lymphoblastoid cell lines from WS patients with typical and atypical deletions and normal controls, a total of 47 genes were differentially expressed between the typical population and the atypical and normal control cells (38). These 47 genes were suggested to explain the highly penetrant features of WS not observed in those with an atypical deletion, with functional enrichment analyses showing an over-representation in ‘glycolysis’ and ‘neuronal migration’. The genes within these pathways include *PFKP*, *PGK1*, *ENO1*, and *MAP1B* and are all either highly or dynamically expressed in brain development.

The second study examined expression profiles in fibroblasts from WS and unaffected individuals to elucidate molecular pathways that may contribute to WS features. Of the 868 identified differentially expressed genes, these authors found significant overrepresentation in major histocompatibility complex genes and gene products that localize to the postsynaptic membrane (39). This study went on to identify modules specific to the

transcriptional profile of WS fibroblasts and compare them to other studies analyzing the transcriptional profile of control fibroblasts. The two modules with strongest enrichment for GO terms highlighted ‘extracellular region’ and ‘extracellular matrix’ biological terms and contained neocortical, developmentally regulated genes such as *NOTCH3*, *SCN1B*, and *CNTNAP1*, which was also previously mentioned to be dysregulated in ASD.

Schizophrenia

As noted above, duplications of the 7q11.23 locus, previously associated with ASD, have also been implicated in schizophrenia (SCZ) (37). Interestingly, although no transcriptional profiling has been done on tissue from patients diagnosed with SCZ and confirmed to have this specific CNV, this association builds on the current general knowledge of CNVs and their role in neurodevelopmental and psychiatric disease. Nevertheless, other transcriptomic-based analyses of tissue from SCZ patients have yielded insight into the molecular mechanisms underlying the disorder, and align with previous studies suggesting abnormal brain development. Targeted measurements of single genes comparing NCX samples from post-mortem SCZ and unaffected patients have identified significant upregulation of *CNIH-1*, *-2*, and *-3* (40), which regulate AMPA receptor trafficking; differential isoform expression of *KCC2* (41); which drives the developmental switch of GABA from excitatory to inhibitory; and decreased *RELN* in dorsolateral prefrontal NCX, which is involved in NCX layer migration and is also implicated in other neurodevelopmental disorders (42).

Other approaches utilizing high-throughput transcriptomics have given a broader perspective of genes and pathways that may be altered in SCZ; however many of these early studies suffered from small cohort sizes or methodological differences that made concrete conclusions difficult. Two independent studies sought to combine and meta-analyze these multiple transcriptomic studies to bring clarity to genes and pathways affected in the SCZ brain. Perez-Santiago *et al.* identified 144 genes to be differentially expressed in the dorsolateral prefrontal cortex of SCZ vs. unaffected control samples, 74 of them ‘novel’ due to the increased statistical power of the meta-analysis. Genes most profoundly affected include *SI00A8*, a calcium-binding protein involved in cell-cycle progression and differentiation; *EGR1* and *EGR4*, transcriptional regulators that are responsive to nerve growth factor; and *NEUROD6*, an upstream regulator of neural differentiation (43). A similar meta-analysis by Mistry *et al.* identified 95 differentially expressed genes, and though five of the datasets overlapped with those analyzed by Perez-Santiago *et al.*, there was surprisingly little overlap of differentially expressed genes, which is likely a reflection of different experimental approaches (44). Nevertheless, genes identified to be differentially expressed by Mistry and colleagues did categorize into ‘cell adhesion’ and ‘transcriptional regulation,’ processes imperative to establish a normally functioning brain. Together, these two meta-analyses hint at potential mechanisms for SCZ and set the stage for additional transcriptomic studies to enhance the picture on mechanisms of SCZ.

More recently, exon-array comparative analysis identified 43 genes displaying differential expression within prefrontal NCX, and 341 genes in prefrontal NCX and the caudate in SCZ cases versus controls (45). Of these, alternative exon-usage was highlighted for *ENAH*, previously implicated in SCZ (46), and *CPNE3*, which is a calcium-dependent, membrane-

binding protein thought to function during cell migration. In a similar study, RNA-seq of the superior temporal gyrus from SCZ patients identified 772 genes significantly changed from controls, which included genes identified from previous array-based studies, including *EGR4* and those categorized into ‘neural development’ GO (47). This study also identified more than 2000 genes exhibiting differential promoter usage and over 1000 genes with differential splicing, with many of these genes directly relevant to neurological function, such as *GABRA5A*, *MBP*, *SYP*, and *SYT1*. These transcriptome studies on SCZ largely contribute to the hypothesis that abnormal neurodevelopment facilitates psychiatric disease and that the differences are in the specific functions of individual genes. However, like ASD, a stronger understanding of the spatial and temporal characteristics of SCZ development is needed – and similarly to the recent breakthroughs in ASD, new advances have been made in this regard by combining gene discovery with the available human developmental transcriptome data.

In the first study to integrate whole-exome sequencing in SCZ alongside human brain transcriptome analysis, Xu *et al.* (48) demonstrated an excess of ‘functional’ *de novo* mutations in SCZ and then characterized their expression pattern in the human brain using the BrainSpan dataset (5). Narrowing their analysis to two regions previously implicated in SCZ, the dorsolateral prefrontal cortex and hippocampus, the authors assessed effect size by expression pattern and observed the greatest effect when considering the set of genes most highly expressed during early and midfetal development. Shortly after this study, Gilman *et al.* (49) utilized a computational approach, NETBAG+, to perform an integrated analysis of several whole-genome data sets identifying specific SCZ-associated variants, including *de novo* SNVs implicated by Xu *et al.* After identifying several gene networks enriched for GO terms such as ‘axon guidance,’ ‘neuronal cell mobility,’ ‘synaptic function,’ and ‘chromosomal remodeling,’ the authors assessed the expression of network genes with BrainSpan (5) and similarly demonstrated that these genes are highly brain expressed, with strongest expression during prenatal development. Nevertheless, like the initial attempts in ASD, limited temporal and spatial resolution was achieved.

However, in a recent whole-exome sequencing study in simplex SCZ families, Gulsuner and colleagues (35) identified 54 genes potentially carrying risk for the disorder based on the presence of at least one rare *de novo* mutation. These point mutations putatively influencing gene function were then evaluated with regard to their localization using the BrainSpan RNA-seq based developmental transcriptome (www.brainspan.org). These expression profiles were clustered into four brain regions and three time periods, expression correlation coefficients for these 54 genes were calculated to identify networks of connected genes. The network with the highest level of interconnectedness occurred in the fetal time period and frontal NCX region, suggesting this is an important point of spatial and temporal convergence of SCZ genes. Similarly to the studies conducted with ASD-associated variants, these analyses implicate both prenatal development and the frontal NCX in SCZ, suggesting that perturbations of key developmental networks in the frontal NCX may increase risk for both ASD and SCZ (Figure 2B) – a result consistent with shared genetic etiology (50).

Future directions in utilizing the human brain transcriptome to understand the biology of neurodevelopmental disorders

Although the recent studies outlined above have made considerable inroads into the biological underpinnings of psychiatric and neurological disorders, many questions remain. For instance, the specific perturbations within these time periods and the mechanism of pathogenesis are still unclear. Moreover, so far, these approaches implicate similar brain regions and time periods in the pathogenesis of both ASD and SCZ; consequently, additional studies will be needed to elucidate the key differences. Along these lines, higher resolution gene expression data in terms of time period, brain region, cell type, and transcript isoform usage are needed to better dissect these neurodevelopmental disorders in isolation, as well as to understand commonalities and differences between them. Finally, expansion of these methods and data to other complex neurodevelopmental disorders such as Tourette syndrome is necessary.

Conclusion

Next-generation sequencing has greatly facilitated the ability to discover novel genetic causes of neurodevelopmental disorders as well as to characterize ‘healthy’ and ‘pathogenic’ transcriptomes. Studies that integrate gene discovery and transcriptome profiling in a data-driven manner make the critical step of identifying when and where to study psychiatric and neurological disorders, and are examples for other complex neurodevelopmental disorders. Once we understand how and why specific genes contribute to these diseases, we will be able approach the most important hurdle of identifying effective therapeutic intervention.

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Key Points

- Gene expression patterns in the developing human brain are highly dynamic and reflect the underlying biological processes
- Transcriptome studies of human post-mortem tissues from affected and unaffected donors identify candidate mechanisms for abnormal brain development.
- Genetic mutations perturbing neurodevelopmental processes are known to result in psychiatric or neurological disorders. Recent whole-exome studies have identified variants in genes displaying a much wider array of functional diversity.
- Expression patterns of the diverse genes disrupted in complex neurodevelopmental disorders in the developing human brain give a fresh perspective on the underlying biology.

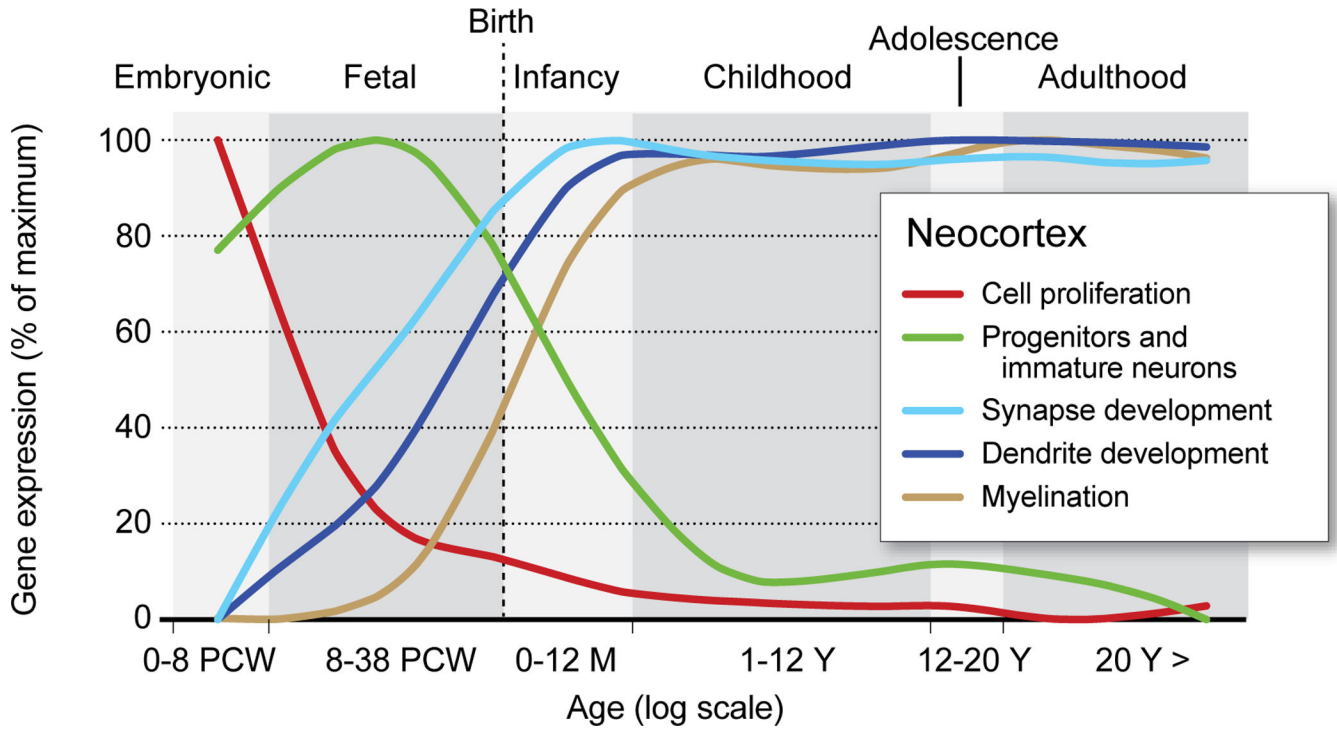


Figure 1. Timeline of major human neurodevelopmental processes based on gene expression trajectories

Expression trajectories of genes associated with major neurodevelopmental processes reflect the occurrence and progression of these processes in the human neocortex (NCX). The expression levels and trajectories have been adopted from Kang et al. (5). These trajectories suggest that the prenatal development is the most dynamic phase of the human brain development. PCW, post-conceptual weeks; M, months; Y, years.

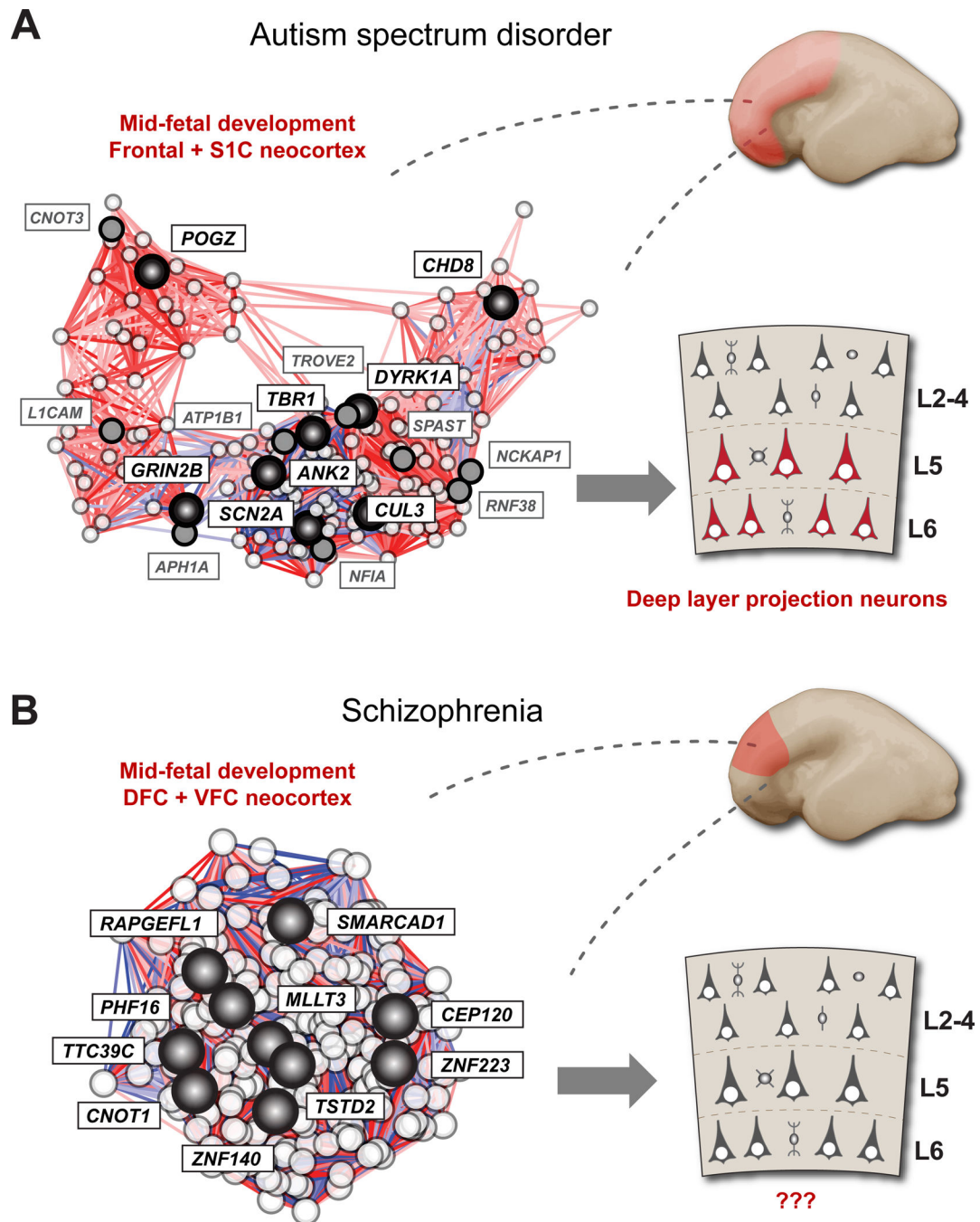


Figure 2. Spatio-temporal resolution of autism spectrum disorder and schizophrenia pathology

A. Autism spectrum disorder (ASD) associated genes converge in deep layer cortical projection neurons in prefrontal and primary motor-somatosensory cortical regions during midfetal development (Willsey et al., 2013). The two midfetal networks from Willsey et al. were combined into one network encompassing all of mid-fetal development (10–24 PCW). High confidence ASD genes are in black, probable ASD genes are in grey, and other co-expressed genes are in white; positive and negative correlations are signified by red and blue, respectively. Adapted from (34). B. Potential schizophrenia risk genes are highly

interconnected in the frontal NCX, particularly in dorsolateral and ventrolateral prefrontal cortical regions (DFC and VFC, respectively), during fetal development (35). Starting with the genes from the DFC and VFC gene network identified by Gulsuner and colleagues, co-expression network analysis built a network of 841 genes highly co-expressed during fetal development (13–26 PCW). These genes were ranked by connectivity and the top 25% (210 genes) are plotted here, with the ten most connected genes in black.