**Title:** Integrative Functional Genomic Analysis of the Developing Human Brain

**Alt**: Integrative Functional Genomic Analysis of Human Brain Development and Neuropsychiatric Etiologies

**Authors:** LIST OF KEY CONTRIBUTORS …. BrainSpan Consortium +, PsychENCODE Consortium: Developmental Subgroup ++, …. LIST OF KEY SENIOR AUTHORS

(~ 30 authors, all others could be listed at the end of the paper)

**Affiliations:**

+ The consortium authors are listed at the end of the paper, with authors who are listed above removed.

\* These authors contributed equally to this work

# Correspondence to: xxxx

**Abstract:** 125 words

To broaden our understanding of human neurodevelopment, we profiled the transcriptomic and epigenomic landscapes across brain regions and cell types for the entire span of prenatal and postnatal neurodevelopment. Integrative analysis of multimodal data revealed temporal, regional, sex and cell type specific dynamics. Notably, we observed a global transcriptomic and epigenomic transition beginning during late fetal development, corresponding to sharply decreased intra- and inter-regional differences, and changes in cellular composition and maturational states. Genes associated with neuropsychiatric disorders and traits converged in a small number of co-expression modules, with *MEF2C* and *TCF4* showing a particularly high overlap. Modules also revealed key relationships between epigenetic regulation, gene expression, and neurodevelopment. We revealed novel insights into brain development and neuropsychiatric disorders, and provide a foundational resource for future research.

**Introduction**

The development of the human brain is a highly complex process that unfolds over several decades and is reliant on the diversity and precise spatiotemporal regulation of the transcriptome (PMID: 28661727; PMID: 26796689). Additionally, there is increasingly persuasive evidence that dysregulation of the transcriptional, regulatory, and epigenetic processes underlying the spatial and temporal progression of neurodevelopment can have dire consequences for brain function (PMID: 26796689; PMID: 26404826; PMID: 26924435; PMID: 25710529; PMID: 24183011; PMID: 26361314; PMID: 21068826; PMID: 1838531; PMID: 24267886). However, many of the regulatory and epigenomic features governing the transcriptome of the developing human brain remain unknown and may be specific to defined biological contexts in humans or closely related primate species (PMID: 21729779; PMID: 25710529). As such, it is difficult or impossible to identify or study the full roster of functional genomic elements using most common model organisms or cell culture systems (PMID: 26796689; PMID: 24183016; PMID: 29437890). On the other hand, for reasons of scale and challenges of developmental human tissue availability, our understanding of different facets of the transcriptional, epigenetic, and regulatory architecture of the prenatal and postnatal developing human brain remains highly incomplete (PMID: 19477152; PMID: 19307592; PMID: 24695229; PMID: 29217575, PMID: 25734491; PMID: 25745175; PMID: 25721503; PMID: 29307494; PMID: 27667684).

**Data generation and cross-modal integration**

In the present report, we describe the generation and integrated analysis of multiple genomic data modalities (i.e., transcriptional profile, methylation status, histone modifications, CTCF binding sites, and genotype) generated on dissected bulk tissue samples or at the single cell level from a total of 58 de-identified post-mortem brains obtained from clinically and histopathologically unremarkable donors of both sexes and various ancestries, ranging in age from 5 postconceptional weeks (pcw) to 40 years (fig. 1A). Genotyping of donors’ brain DNA using a HumanOmni2.5-8 BeadChip revealed no obvious genomic abnormalities in any of the brain specimens and we observed that their genetic diversity varied in a random way (Supplementary materials).

For transcriptome analysis, tissue-level mRNA-seq was performed on a total of 607 histologically verified high-quality tissue samples representing 16 anatomical brain regions (11 neocortical areas (NCX), hippocampus (HIP), amygdala (AMY), striatum (STR), mediodorsal nucleus of thalamus (MD) and cerebellar cortex (CBC)) involved in higher-order cognition and behavior (figs. 2A and S1; Supplementary materials). These regions were systematically dissected from 41 brains ranging in age from 8 pcw to 40 postnatal years (18 females and 23 males; postmortem interval (PMI) = 12.9 ± 10.4 hours; tissue pH = 6.5 ± 0.3; RNA integrity number = 8.8 ± 1) (fig. 1 and table S1). Due to the limited size of prenatal samples, small RNA sequencing (smRNA-seq) was performed on 278 samples covering these 16 regions from 22 postnatal brains (fig.1 and table S2). These tissue-level RNA-seq analyses were complemented by single-cell RNA-seq (scRNA-seq) data generated from 1,074 cells that passed quality control measures and were collected from embryonic and mid-fetal fronto-parietal neocortical wall of an independent set of 9 brains ranging in age from 5 to 20 pcw (fig. 1 and table S3). For epigenome analysis, DNA methylation data was generated for 269 postnatal samples covering the same 16 brain regions analyzed by RNA-seq (Fig. 1 and table S4). Additional epigenomic data was generated for several histone marks (H3K4me3, H3K27ac, and H3K27me3) and the binding sites for the epigenetic regulatory protein CTCF, which together identify a large fraction of active enhancers, promoters, and repressors, as well as insulators. These data were generated from a subset of samples taken from dorsolateral prefrontal cortex (DFC aka DLPFC) and CBC from midfetal, infant, and adult brains (Fig. 1 and table S5).

RNA-seq datasets underwent stringent quality control and normalization procedures (fig. S2 to S6; Supplementary materials) to alleviate the influence of confounding effects (e.g., post-mortem interval, tissue pH, RNA integrity number, batch, 3’ bias). To optimize statistical power, we grouped specimens into 9 time windows (w1-w9) based on major neurodevelopmental milestones and agnostic transcriptome-based temporal spacing of constituent specimens (fig. 1A and table S1). *In silico* pooling of all mRNA-seq samples resulted in the detection, at RPKM>=1, of 79.7% (15,845/19,881) of protein-coding genes, indicating that a large number of mRNA genes are expressed in the developing and young adult human brain (fig. S4A). In addition, 2% of reads were from intergenic regions (fig. S4C and table S1) that may represent novel transcriptionally active regions (fig. S7 and table S6), including unannotated 5’ and 3’ UTRs. Moreover, we found most genes were temporally (67.8%, 13,485/19,881) or spatially (54.5%, 10,825/19,881) differentially expressed between at least two windows or regions respectively, while 52.2% (10,371/19,881) of genes were both concurrent, indicating the majority of spatially differentially expressed genes (95.8%; 10,371/10,825) were also temporally differentially expressed (fig. S8). This was consistently observed in weighted gene co-expression network analysis (WGCNA), of which numerous (x of 73) modules exhibited temporal (non-spatial) differential expression and only a few modules (x of 73) encompassing a relatively small number of genes exhibited strictly spatial (non-temporal) differential expression (fig. S9 and table S7). smRNA-seq similarly revealed dynamic spatio-temporal expression of small RNAs, with only 289 of 1,207 detected mature miRNAs, as well as several Y-RNAs, snRNAs, and snoRNAs, expressed (RPM >=1) across all 278 samples. tRNAs were more consistently expressed, with 93% detected in at least one sample and 75% detected across all samples.

We applied a clustering and classification iterative algorithm (Lake et al., 2016) to scRNA-seq data following an initial division of the dataset based on the age (i.e. embryonic or fetal) of the donor brain. This led to the unbiased identification of 24 clusters representing distinct types of neural and non-neural progenitor and postmitotic cells (fig. S9 to S10). Differential expression analysis with subsequent specificity quantification yielded novel gene markers for specific cell types (fig. S11; Supplementary materials) and broad populations of similar cell types (figs. S12 and S13). We aligned our embryonic and fetal single cell clusters with other single cell clusters from mostly adult brains (Darmanis et al. 2015), revealing the developmental similarities for major cell classes and the uniqueness of some prenatal cell populations including intermediate progenitor cells and embryonic or early fetal neurons expressing markers indicative of migration or recent post-mitosis (fig. S14). In addition, more than one cluster in the neuronal lineage retained the expression of proliferative or progenitor gene markers along with early neuronal gene markers, suggesting intermediate progenitor cells or nascent neurons may begin to express neuronal markers prior to or immediately following terminal mitosis (fig. S14). Despite the alignment of neuronal cells, integration with a single-cell dataset of adult neocortical neurons (Lake et al. 2016) revealed that the postmitotic excitatory projection neurons during embryonic to late midfetal development co-expressed a number of gene markers mutually exclusive in their adult counterparts (fig. Sx), indicating that their molecular identities are not fully resolved before the end of late midfetal development and may be plastic during early postmitotic differentiation.

In the epigenomic analyses, DNA methylation profiling revealed that the majority of CpG loci were either highly (37.5%; Beta value ß >= 0.8) or poorly (31.8%; ß <= 0.2) methylated (fig. S14A), and the survey of genomic distribution of CpG loci showed methylation levels were high in genic and intergenic regions but low at transcription start sites (TSSs) (fig. S14B). These methylation sites showed regional differences with CBC differentiated by the first principal component (fig S14C) and presenting the highest values of specificity scores (S14D). Subcortical areas tended to exhibit higher specificity scores than neocortical areas, and, among neocortical areas, V1C was the most distinct (S14D). Alongside of methylation, the characterization of histone modification indicated the majority of H3K4me3 marks were proximal to TSSs while a plurality of the other chromatin modifications and CTCF binding sites occupied intronic or intergenic regions (fig. S15A). Moreover, we noticed the histone modification altered in either temporal or regional dimension, leading to the identification of 11,365 and 5,537 prenatal enriched and 13,147 and 11,788 postnatal-enriched regulatory regions in DFC and CBC, respectively.

Our multiple data modalities, often generated from same region and same donor brain, allowed a unique opportunity to conduct a pure integrative analysis with less genetic confounding effects. As an exemplification, the integration between DNA methylation and complementary mRNA-seq data revealed that the methylation levels across the gene body were independent of gene expression, but the methylation at TSS varied with gene expression level (fig. S17A). Less than 20% methylation occurred at the TSS of well-expressed genes (RPKM >= 1), whereas greater than 50% methylation occurred at the TSS of low expressed genes (RPKM <= 0.1) (fig. S17B). Analogously, the association analysis between histone modification and transcriptomic datasets disclosed the presence of CBC-specific H3K4me3 and H3K27ac marks in the adult human brains correlated strongly with increased gene expression in CBC relative to DFC (fig. S17C,E), vice versa. In the meanwhile, we observed an negative correlation between regionally-specific H3K27me3 activity and gene expression in the CBC and DFC (fig. S17D). Taken together, these results demonstrate the possibility to uncover the underlying relationship between gene expression and epigenetic modifications including methylation status and putative regulatory elements (fig. S17).

**Global dynamics of transcriptional signature**

To gain a broad understanding of transcriptional variation across brain development, we analyzed the level of similarity between individual samples in the mRNA-seq dataset by multidimensional scaling (MDS). It is shown a clear divide between prenatal (w1-4) and postnatal samples (w6-9), with samples from late mid-fetal in the third trimester (w5) generally spanning this divide (fig. 2B, S18). To determine the relationship among these three groups, we performed unsupervised circular hierarchical clustering analysis and found these samples from w5 were more similar to early postnatal samples rather than late mid-fetal samples (fig. 2C), suggestive of a major transition that begins prior to birth and appearing to mark a global shift between early and late developmental transcriptional programs. This transition was also apparent at the intra-regional level, as eleven neocortical areal transcriptomes correlated well across both early and mid-fetal (w1-4) and late postnatal (w6-w9) phases, but displayed a sharp decrease in correlation around late fetal development and early infancy (w5) (fig. 2D). This trend was also apparent for the CBC (fig. 2D) and the other brain regions assessed (fig. S19). Measurements of alternative splicing also revealed a similar divide (fig. S20), indicating that the sharp later fetal transition is a broad transcriptomic phenomenon.

To further explore how the spatial dynamics of the human brain transcriptome changes with age, we applied our novel principal component analysis algorithm (AC-PCA; REF) that adjusts for inter-individual variations (REF). Within any given developmental window, AC-PCA constantly exhibited a clear separation of brain regions, but the average dissimilarity between transcription profiles of brain regions declines with age before birth and increases with age after birth, with CBC accounts for most of the dissimilarity (**Fig. 2E, 2F and fig. S21**). This implied the correspondence between transcriptional signature and the developmental origin, because the dorsal pallium-derived structures of the cerebrum (i.e., NCX, HIP, and AMY) becomes increasingly similar across prenatal development but the subcortical regions (CBC and MD) tend to be more distinct after birth. To quantitatively measure the influence of any brain region, the exclusion of any one region from AC-PCA demonstrated the distinct development trajectories of subcortical regions and simultaneously exhibited a hourglass-like pattern with the transition site precisely occured at late fetal period and early infancy (w5) for six brain regions (**Fig. 2F**). Lastly, it is worth to note that the same analyses performed by using exon array dataset provided the similar results (**fig. Sx**).

Analysis of differential expression, both at the level of gene and isoform transcript, additionally supported the existence of a transition beginning during mid-to-late fetal development. Pairwise comparisons of gene expression across all 16 brain regions found a reduction in the number of genes differentially expressed across brain regions during window 5 (fig. S19). It was also proven by the dramatic drop in the transcriptional change rate between w5 and w6 (**fig. S22**). On the other hand, the number of genes exhibiting regional differences in alternative splicing (**fig S23)**, as well as the global measure of alternative splicing such as exon PSI, are significantly higher during prenatal than postnatal ages (**fig S20 and Supplementary Materials**). And the gene expression of 68 RNA-binding proteins implicated in splicing events are expressed more highly in all brain regions at prenatal ages than at postnatal ages with the transition site at window 5 (**fig. S24**).

**Deconvolution of cellular complexity and dynamics**

The same inter-regional hourglass pattern was observed in different regions of the developing neocortex, with greater dissimilarity across regions at early ages (**fig. Sx2, Fig. 3A**). Prefrontal areas were most distinct with a general rostrocaudal pattern of variation during mid-fetal periods. At later ages, adult patterning closely resembled the overall topography of the adult human cerebral cortex as described previously (Hawrylycz et al., 2012), with proximal areas showing the greatest transcriptional similarity and V1C showing the most distinct patterning (**Fig. 3A**). Across the entire development, the similarity between cortical regions (mean distance in the first two PCs) showed a pronounced decrease centered on the late fetal and early infancy samples of w5 (**Fig. 3A, fig. S**).

The high inter-regional variation observed during prenatal ages corresponds to ages relevant for several neurodevelopmental disorders but also refractory to analysis for ethical and practical reasons inherent to the use of human prenatal tissue. To help understand the mid-to-late fetal transition, taking into consideration the cellular complexity and dynamics of the developing human brain, we deconvolved tissue-level RNA-seq data using prenatal and adult (Lake et al., 2016) single cell RNA-seq data to assess changes in cell proportions and maturational states across development. As expected, neural progenitor cells comprised a progressively lesser proportion of the total cellular population (**Fig. 3B**) while the glial and endothelial cell portions rose across prenatal ages (**fig. S**). Adult and fetal signatures of excitatory and inhibitory neurons were clearly distinct, with fetal signatures for both decreasing across the mid-to-late fetal transition while adult signatures rose (**Fig. 3B**). Markers specific for prenatal astrocytes increased across prenatal ages. Conversely, the expression of adult astrocytic markers (from REF) rose during late midfetal periods (Fig. 3B, **fig S25**), as did markers for biological processes including dendrite formation, synaptogenesis, and myelination (**fig. S26**). The expression of RNA binding proteins responsible for RNA-splicing also decreased across the late mid-to-late fetal transition, corresponding to the commensurate increase in non-neuronal populations (**fig. S24**) and consistent with the decreased expression of RNA-binding proteins in mature neuronal populations (fig. S25).

We next reasoned that the observed mid-to-late fetal transition in gene expression could result from spatiotemporal variation in cell type proportions or biological processes. To address this possibility, we calculated the variance in cell proportions among neo-cortical regions and observed that the pattern of variance through time mirrors the developmental hourglass (**Fig 3C**). Driven by heightened regional differences in the proportions of neural progenitors and embryonic and fetal neurons relative to postnatal cell populations, we found high levels of inter-regional variation during embryonic and early fetal periods followed by a mid-to-late fetal transition and reduced variation during early postnatal development.

Interregional variation in the expression of genes associated with progenitor cell division, neuronal maturation, laminar specification, myelination, oligodendrocytes, and astrocytes exhibited a similar pattern, with neuronal processes including maturation and laminar specification showing high levels of variation during fetal periods followed again by a mid-to-late fetal transition (**fig. S26**).

**Spatiotemporal and multimodal integration**

Further evidence that cellular dynamics underlay the mid-to-late fetal transition came from weighted gene co-expression network analysis and analysis of cell type- and spatiotemporal-specific patterns of epigenetic regulation. WGCNA determined 73 gene-coexpression modules that we organized according to their spatio-temporal dynamics. We found 44 modules showing regional expression differences among areas in the brain (spatial) and 40 modules showing expression differences between pre-natal and post-natal neocortical areas (temporal). 27 modules were spatial and temporal, whereas 16 modules were neither spatial nor temporally dynamic (**Fig 4A**). Spatiotemporally dynamic modules significantly exhibit the greatest change in neocortical expression across window 2 or 5 (eg. ME2, 10, 32, 37). These modules were enriched for neuronal genes, genes associated with putative fetal-active enhancers, and/or genes associated with undermethylated sites in NeuN+ versus NeuN- cells (NUM sites) (which we called Neuronal or “N”-Type modules/associations in **Fig. 4A; fig. S27**).

Conversely, genes associated with adult-active enhancers, methylation sites undermethylated in non-NeuN-positive cells (non-NUM sites) and glial genes (Glial or “G”-Type modules/associations in **Fig. 4A; S27**) were co-enriched in modules where neocortical gene expression exhibited the greatest change as an increase across the mid-to-late fetal transition (eg. M3, 7, 13, 56). G-type associations were enriched in modules where temporal, but not spatial, specificity was observed. This observation is in agreement with a recent study documenting increased spatial diversity of neuronal cell types relative to glial cell populations (Ed Lein REF).

Analysis of sexual dimorphism revealed that modules enriched for genes exhibiting sexual dimorphism were enriched in modules with no spatial or temporal differential expression, as defined above. Yet several modules enriched for sexually dimorphic genes showed no regional differences but were largely distinct between prenatal/perinatal and postnatal ages, with changes in sexual dimorphism across the mid-to-late fetal transition.

Associations between gene expression, methylation status, and enhancer activity also occurred at the level of individual enhancers, methylation sites, and genes, with commensurate effects on specific cell types and developmental programs. Putative fetal-active and adult-active enhancers were associated with higher fetal or adult gene expression and were enriched in neuron-specific and glial-specific genes, respectively (see Supplementary Materials) (**Fig. 4B, S31**). In addition, fetal enhancers were enriched for methylation sites that were progressively more methylated across postnatal ages (post-up) while adult-active enhancers were enriched for methylation sites that were progressively less methylated across postnatal ages (post-down) (see Supplemental Materials) (**Fig. 4C, S27**). Both post-up and post-down sites were themselves depleted at transcription start sites (TSSs), enriched for NUM and non-NUM sites (**S31**), and enriched for fetal and adult enhancers, respectively (**Fig. 4B**). Post-up sites were also enriched among neuron-specific genes and glial genes while post-down sites were enriched in glial genes (**Fig. 4B**). Further suggesting a relationship between enhancer activity, methylation, and cell type, both genes associated with fetal-active enhancers as well as those associated with post-up sites (see Supplemental Materials) were enriched for gene ontology annotations related to early events in neural development, such as neurogenesis, cell differentiation, and synaptic transmission, but not for processes generally occurring later in development including glial cell development, oligodendrocyte development, and myelination (**Fig. 4B,C, S27**). In contrast, genes near adult-active enhancers and post-down sites exhibited a largely complementary pattern of enrichments (**Fig. 4B,C, S27**).

**Cellular and temporal convergence of disease risk**

Loci implicated in several neurodevelopmental disorders have been identified through genome-wide association studies (GWAS) and are enriched in putative noncoding regulatory elements (PMID: 25544106). We sought to apply our dataset to determine whether the cis-regulatory elements we identified were implicated in neurological traits (such as IQ and neuroticism) or disorders with different ages of onset (including ASD, Alzheimer’s disease, major depression disorder, and schizophrenia) and whether genes associated with these regulatory elements converged on discrete cell types or were enriched in distinct co-expression modules. We found that loci implicated in schizophrenia, MDD, IQ, neuroticism, and ASD were almost exclusively enriched in DFC-specific, and not CBC-specific, regulatory elements, with no evidence for temporal specificity. On the contrary, loci implicated in ADHD were enriched exclusively in fetal CBC regulatory elements (**Fig. 5A; fig. S28, Supplementary Materials**) and non-neurological traits were not enriched in any set of regulatory elements analyzed.

After aggregating GWAS SNPs and identifying candidate associated regions based on their P-values and linkage disequilibrium patterns in CEU individuals (REF), we next identified disease risk-associated genes by leveraging Hi-C data derived from developmental fetal and adult brains (REF, REF) and H3K27ac activity in fetal DFC (**Fig. ??**). We supplemented these lists of disease risk genes with a list of high-confidence ASD risk genes (Sanders et al., Neuron). We found that disease risk genes converged on a small number of coexpression modules (**Fig. 5B**), all of which exhibited temporal specificity in the neocortex (**Fig. 5B**). Moreover, eigengenes for seven of the eight modules enriched for disease-associated genes exhibited their greatest expression during embryonic or fetal periods; Module 12, which was enriched for genes we associated with AD, was the lone disease-associated module whose eigengene exhibited its greatest expression during postnatal periods. We observed N-type signatures for six of eight disease-associated modules, while Module 12 was the lone module associated with astrocytic gene expression and adult-active enhancers (**Fig. 5B**).

Module 37 was enriched for genes associated with neuron undermethylated sites and fetal enhancers and whose expression was enriched in neurons. Module 37 was also exceptional for its disease association, being enriched for genes associated with ASD, SCZ, IQ, and neuroticism but not non-neurological characteristics such as height or a diabetes-related trait (**Fig. 5B**). Complementary module-based association analysis with MAGMA, testing for an enrichment in association to disease around TSSs of genes in any given module, confirmed enrichment for SCZ, IQ, and neuroticism in Module 37 (**Fig. 5B**). We investigated the signal overlap at the gene level and found that multiple genes in Module 37 were associated with up to four different traits, including MEF2C, a well-known disease-risk gene associated with SCZ, ASD, and neuroticism (**Fig. 5C**). Other key developmental genes such as TCF4 and SATB1 were also associated with multiple disorders, and these genes, as well as MEF2C, were among the most connected gene in an integrated interaction network (GeneMANIA REF) (**Fig. 5C,D**).

**Discussion**

In this study, we have presented what is, to our knowledge, the most comprehensive multi-platform functional genomic analysis of the developing human brain. Data generated include information about the transcriptome, at both the gene or isoform level, from bulk tissue and single cells, and regulatory modalities including methylation and histone modification. The presence of these multiple data modalities in a unified resource, and largely from the same tissue samples, allows the integration of information to an extent not typically possible in isolated studies spanning prenatal and postnatal human neurodevelopment.

While transcriptional differences between distinct brain regions remain robust across development, the global brain transcriptome exhibits a sharp transition beginning during mid-to-late fetal development. Underlying this transition there is an ‘hourglass’ shaped pattern of variation with high prenatal interregional and interareal variation that decreases across late fetal ages, and recovers somewhat beginning in late childhood and continuing through adulthood. Our accompanying analysis of the spatio-temporal transcriptome of the macaque brain development (Zhu et al.) reveals that this transition and pattern is likely conserved across the catarrhine group of primates.

Intriguingly, principal component analysis, unsupervised hierarchical clustering, multidimensional scaling, and analysis of differential expression all suggest this transition begins well prior to birth. In particular, multidimensional scaling and hierarchical clustering suggest that late fetal samples are more closely associated with postnatal samples than with other prenatal samples, a surprising discovery as the two late fetal samples (35 and 37 PCW) were closer in age to the three nearest late mid-fetal samples (91 to 112-day separation) than to the three nearest postnatal samples (129 to 143-day separation). Second, biological processes active in the human brain and common across multiple regions exhibit spatial diversity during prenatal ages and late postnatal windows but not during perinatal periods and early infancy following the mid-to-late fetal transition. This likely reflects spatiotemporal gradients of expression during prenatal ages and functional divisions between mature neocortical areas and brain regions in the adult. Third, we found that the prolonged development of neural, particularly neuronal, cell types in the human brain extends throughout prenatal development and into the mid-to-late fetal transition; by 20 PCW, many neurons in the neocortex have not yet begun expressing markers indicative of mature cell types or laminar position, have not extended processes, and have not begun to express synaptic or other markers associated with cell maturity. In addition, deconvolving tissue-level data with these single cell data demonstrated the rise of mature neuronal molecular signatures during late fetal development and into infancy (**xx pcw – yy pcw**). Cell-type deconvolution also reveals that the dynamic inter-regional distance in genes expression correlates with the inter-regional variation in the relative proportion of cell-types. The mid-to-late fetal transition may therefore represent an inflection point where developmental and spatiotemporal variation in human neocortical development is transiently consolidated in advance of the emergence of functional differences between adult brain regions.

To go beyond the information each data type reveals about the human brain transcriptome, we performed integrated analyses of all our data modalities through gene co-expression modules. We identified modules with or without spatiotemporal dynamics enriched in distinct epigenomic features and cell-types. This approach also permitted the identification and characterization of modules associated to neurological traits. Of particular interest is module 37, in which putative risk genes for ASD, schizophrenia, IQ and neuroticism converge. Of those genes, *MEF2C* and *TCF4* are present in ASD, schizophrenia and neuroticism, and *MEF2C* also in ADHD. Mutations in *MEF2C* or *TCF4* can result in mental retardation in humans and has previously been associated with several neurological traits and disorders. Recently, it has been reported that *MEF2C* can enhance cognition when overexpressed in adult PFC in mice (PMID:28115742) and that *TCF4* controls neuronal migration in the cerebral cortex (PMID:27752241). Taken together, these observations demonstrate the utility of this resource for integrated analysis of brain development, function, and disorders.

**Acknowledgements:**