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Genetic Control of Expression and Splicing in Developing Human Brain Informs Disease Mechanisms

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SUMMARY

Tissue-specific regulatory regions harbor substantial genetic risk for disease. Because brain development is a critical epoch for neuropsychiatric disease susceptibility, we characterized the genetic control of the transcriptome in 201 mid-gestational human brains, identifying 7,962 expression quantitative trait loci (eQTL) and 4,635 spliceQTL (sQTL), including several thousand prenatal-specific regulatory regions. We show that significant genetic liability for neuropsychiatric

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AUTHOR CONTRIBUTIONS

L.d.I.T.-U., J.L.S., and D.H.G. designed the study. D.H.G. oversaw all analyses and supervised the study. L.d.I.T.-U. and J.L.S. performed sample collection and brain dissections, genotyping, and RNA-seq library preparation. R.L.W. processed and managed the data, performed eQTL analysis, interpreted results, and performed integration with other expression data, GWAS, and network analysis, with input from G.R., C.H., M.J.G., N.M., and B.P. N.M. performed TWASfine mapping. R.L.W. and D.H.G. wrote and prepared the manuscript, with feedback from all authors.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cell.2019.09.021>.

DECLARATION OF INTERESTS

The authors declare no competing interests.

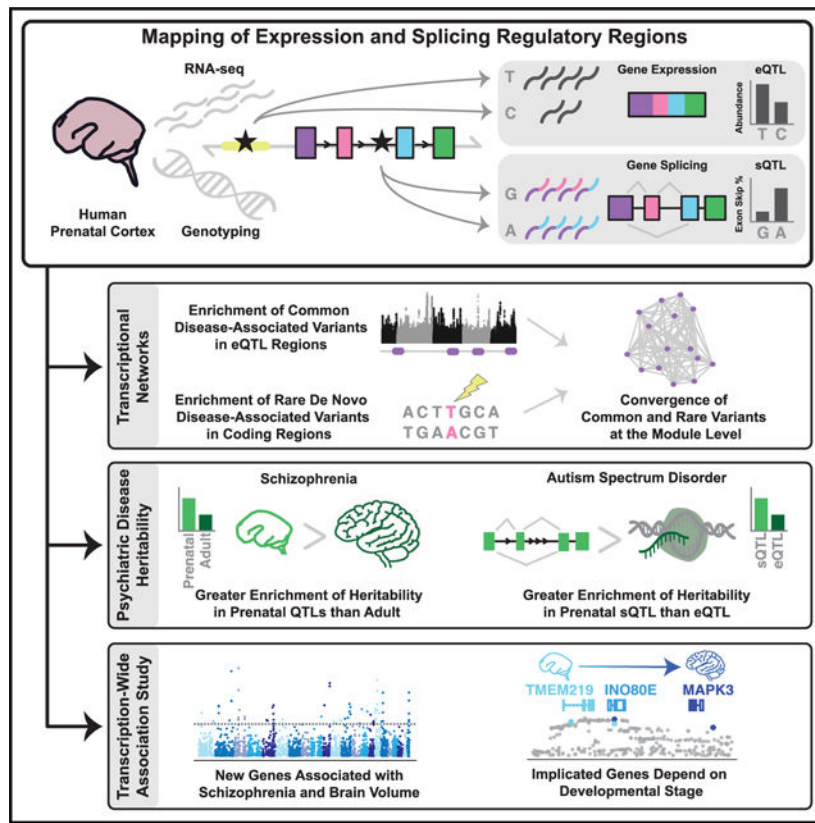
disease lies within prenatal eQTL and sQTL. Integration of eQTL and sQTL with genome-wide association studies (GWAS) via transcriptome-wide association identified dozens of novel candidate risk genes, highlighting shared and stage-specific mechanisms in schizophrenia (SCZ). Gene network analysis revealed that SCZ and autism spectrum disorder (ASD) affect distinct developmental gene co-expression modules. Yet, in each disorder, common and rare genetic variation converges within modules, which in ASD implicates superficial cortical neurons. More broadly, these data, available as a web browser and our analyses, demonstrate the genetic mechanisms by which developmental events have a widespread influence on adult anatomical and behavioral phenotypes.

In Brief

An atlas of expression and splice quantitative trait loci from mid-gestational human brain is integrated with genetic risk for schizophrenia, suggesting additional causal genes and highlighting the importance of QTL datasets derived from developmental stages most relevant to disease initiation.

Graphical Abstract

Graphical Abstract



INTRODUCTION

Neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder (ASD) and schizophrenia (SCZ), are highly heritable, complex conditions (Geschwind and Flint, 2015; Polderman et al., 2015), with hundreds of risk loci identified through large-scale genomic studies (Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium, 2017; Gratten et al., 2014; Pardiñas et al., 2018). However, the ability to interpret these variants has been hampered because many fall in non-coding regions of the genome or in regions of high linkage disequilibrium, making it challenging to identify causal mutations and their functional impact (Gandal et al., 2016; Nica and Dermitzakis, 2013; Schaid et al., 2018). Given the non-coding nature of the majority of these variants, as well as their enrichment in known regulatory regions (Cockerill, 2011; de la Torre-Ubieta et al., 2018), conserved regions (Siepel et al., 2005), and signature histone modifications (Schaub et al., 2012; Visel et al., 2009), many likely function through the regulation of gene expression and splicing (Li et al., 2016; Maurano et al., 2012; Ward and Kellis, 2012). Multiple large-scale projects, including Roadmap Epigenomics, GTEx, and Encode (Ernst et al., 2011; Roadmap Epigenomics Consortium et al., 2015; GTEx Consortium, 2015) have annotated regulatory regions across human tissues. However, little is known about how human allelic variation affects regulatory interactions during brain development, a crucial period for building the scaffold for human higher cognition and brain evolution (Geschwind and Rakic, 2013; Nord et al., 2015; Ward and Kellis, 2012).

Expression quantitative trait loci (eQTL) analysis identifies genetic loci that regulate gene expression. Critically, eQTL relationships are highly dependent on cell type and developmental stage (Dimas et al., 2009; Gerrits et al., 2009; Nica et al., 2011; GTEx Consortium, 2015), consistent with transcriptional surveys of brain development that show prominent temporal changes in gene expression (Colantuoni et al., 2011; Kang et al., 2011; Sunkin et al., 2013). Previous studies suggest that genetic disruption of these patterns, particularly during cortical development, increases risk for developmental and psychiatric disorders, highlighting the need to map regulatory variation within this critical time point (Gilman et al., 2012; Gulsuner et al., 2013; Parikshak et al., 2013; Willsey et al., 2013). However, prenatal brain eQTL analyses based on RNA sequencing (RNA-seq) are relatively small (Jaffe et al., 2018; O'Brien et al., 2018) and none include analysis of spliceQTL (sQTL) (Fromer et al., 2016; Ramasamy et al., 2014; Takata et al., 2017; GTEx Consortium, 2015).

Here, we performed a well-powered eQTL and sQTL analysis in developing human cortex to understand how functional genetic variation impacts phenotypes that likely originate in utero or early postnatal life (Hannon et al., 2016; Jaffe et al., 2016; Parikshak et al., 2015; Weinberger, 1987). We focused on mid-gestation, an epoch that captures neural progenitor proliferation, neurogenesis, and migration (Geschwind and Rakic, 2013; Johnson et al., 2009; Silbereis et al., 2016). We contrasted the genetic control of prenatal and adult expression, which show substantial differences. We found that both eQTL and sQTL contribute substantially to disease risk, but show mostly non-overlapping patterns. Integration of eQTL and sQTL with genome-wide association studies (GWAS) via transcriptome wide association identified new putative mechanisms undetected in adult brain

datasets. These data provide insights into genes relevant to developmental disorders and into establishing which aspects of disease risk affect early corticogenesis, as compared to later processes.

RESULTS

We performed high-throughput RNA-seq and high-density genotyping in a set of 233 prenatal brains (Figure 1). After quality control and normalization of gene expression and genotypes (Figure S1; STAR Methods), we obtained a dataset of 15,925 genes and 6.6 million single nucleotide polymorphisms (SNPs) from 201 individuals. Analysis of ancestry indicate the donors in our study to be 40% Mexican, 25% African-American, 14% European-Mexican, 8% admixed of 3 or more ancestries, 6% Chinese, 5% African-American-Mexican, and 2% of European descent (Figure S2; STAR Methods).

Robust Identification of Prenatal Brain eQTLs

We identified *cis*-eQTLs using FastQTL (Ongen et al., 2016), adjusting for known and inferred covariates (Figure S3; STAR Methods) (Leek and Storey, 2007; Mostafavi et al., 2013). We identified 6,546 genes with a *cis*-eQTL (false discovery rate [FDR] <0.05), hereafter referred to as eGenes (Table S1). Conditioning on the most significant SNP (eSNP) permitted identification of an additional 1,416 independent, secondary eQTLs, for a total of 7,962 eQTLs (STAR Methods). We performed the analysis with two alternative methods to ensure that the eQTLs identified were not driven by ancestry differences within our dataset, which demonstrated high reproducibility of eQTLs identified by FastQTL; 94% of eGenes were also detected by EMMAX (Kang et al., 2008), and 84% of eQTLs were significant when a subpopulation meta-analysis was performed (Willer et al., 2010), despite substantially reduced power (Figure S4; STAR Methods).

eQTLs Mark Active Regulatory Regions

Positional enrichment of eSNPs shows that over 20% of significant eQTLs cluster within 10 kb of the transcription start site (TSS) of its eGene (Figures 2B and 2C), concordant with previous studies showing that promoter variants have a large influence on cognate gene expression (Kim et al., 2014; Stranger et al., 2007; Strunz et al., 2018). The overall distribution of eQTLs is consistent with previous work (GTEx Consortium, 2015; Veyrieras et al., 2008), with 70% of eSNPs located within 100 kb of the TSS, as well as a slight upstream bias (~56%) of eQTLs (Figure 2B). We reasoned that eQTL should overlap with regulatory regions defined by other methods (Figure 2A). Indeed, we find eQTLs are significantly enriched within regions of open chromatin identified in prenatal brain (odds ratio [OR] = 4.42, $p < 2.2 \times 10^{-16}$) (Figure 2D) (de la Torre-Ubieta et al., 2018). Distal eQTLs (>10 kb from TSS) are also enriched within 3D chromatin contacts detected at the same stage of brain development (OR = 2.96, $p < 2.2 \times 10^{-16}$) (Figure 2E) (Won et al., 2016) strengthening confidence that eSNPs are in accessible regions and distal eSNPs regulate their target gene (STAR Methods).

We next annotated eSNPs with chromatin state predictions from prenatal brain tissue (Roadmap Epigenomics Consortium et al., 2015) using GREGOR (Schmidt et al., 2015).

As expected, we observed that eSNPs were most significantly enriched in TSSs, promoters, and transcribed regulatory promoter or enhancers (Figures 2F and S5A), providing further evidence that these eQTLs fall in regions functionally relevant to the regulation of gene expression (Ward and Kellis, 2012).

Functional Characterization of eQTLs Reveals Mutation Tolerance and Regulatory Drivers

Because changes in constrained genes are likely to have a higher impact on fecundity than those that are not highly constrained (Samocha et al., 2014), we reasoned that genes impacted by common regulatory variation would also be more tolerant to protein-disrupting variation (Lek et al., 2016). Indeed, comparing eGenes to genes that do not have a significant eQTL, we find that eGenes are more tolerant to loss of function mutations (Wilcoxon rank-sum test $p < 2.2 \times 10^{-16}$) (Figure 2G).

We next examined whether genomic regions tagged by eSNPs are enriched in transcription factor (TF) and DNA binding protein (DBP) binding sites to understand the mechanism by which eQTLs influence gene expression (STAR Methods) (Arbiza et al., 2013; Cotney et al., 2015). We found significant enrichment of eQTL in binding sites for 39 TFs and DBPs (Figure 2H), many with prominent known roles in brain development and patterning, including *ELK4*, *NRF1*, *SMARCC1*, *SMARCC2*, and *CHD8* (Bestman et al., 2015; Durak et al., 2016; Eising et al., 2019; Lai et al., 2001; Ojeda et al., 1999; Preciados et al., 2016).

As a specific example, we chose *CHD8* (fold change [FC] = 2.4, $p = 7.8 \times 10^{-23}$), because of the strong effect of *CHD8* haploinsufficiency on ASD risk (Bernier et al., 2014; Neale et al., 2012; O’Roak et al., 2014; Sanders et al., 2012). We identified an eSNP falling directly within a *CHD8* binding site for the gene serine-racemase (*SRR*) (Figures 2I and 2J (Cotney et al., 2015), which has been shown to play a role in glutamatergic neurotransmission and modulates neuropsychiatric phenotypes (Basu et al., 2009). *CHD8* knockdown in neural progenitors led to significant differential expression of *SRR* (Figure 2K) (Sugathan et al., 2014). Thus, experimental data from prenatal brain and neural progenitors *in vitro* combined with these eQTL data supports the role of *CHD8* and elucidates the mechanism by which *CHD8* likely regulates this gene.

Robust Identification of Prenatal Brain sQTLs

Effects of genetic variation on RNA splicing are predicted to contribute to complex disease risk (Li et al., 2016). Because there has been no systematic analysis of splicing regulation in human prenatal brain, we reasoned that such analysis would enhance understanding of the link between splicing and disease. We identified 92,449 intronic excision clusters using an annotation free approach (Li et al., 2018), which allowed for discovery of alternative exons, an important advantage in the context of brain, for which isoform annotations are incomplete. We performed sQTL discovery (Ongen et al., 2016), identifying 4,635 significant sQTLs (5% FDR) in 2,132 genes (sGenes) (Figure 3A; Table S2). Of the 4,635 significant sQTLs, 3,295 were annotated introns (71%) and 1,255 (27%) were new cryptic introns (STAR Methods).

Genomic Features of sQTL Distinguish Them from eQTL

Positional enrichment of the most significant SNP per sQTL (sSNP) shows clustering around the splice junction, with 42% of sSNPs within 10 kb of the splice junction (Figure 3B), demonstrating that variants proximal to splicing junctions have a large effect. In contrast to eQTL, the majority of sSNPs (64%) lie within the gene body (Figure 3C), consistent with other tissues (Li et al., 2016). sQTLs were also most strongly enriched in promoters and transcribed regions (Figures 3D and S5B) (Lappalainen et al., 2013; Takata et al., 2017).

Identifying Drivers of Prenatal Brain RNA-Splicing

To validate that the identified sQTLs are tagging splicing regulatory regions, we evaluated sSNP enrichment in experimentally determined RNA binding protein (RBP) binding sites (Yang et al., 2015). We found significant enrichments for sSNPs in binding sites for 36 RBPs (Figure 3E; STAR Methods), many of which do not have well characterized roles in CNS function. Among the identified RBPs with known roles in neurodevelopment are *HNRNPH*, *ATXN2*, and *SRRM4* (Almaguer-Mederos et al., 2018; Grammatikakis et al., 2016; Wang et al., 2007; Zhang et al., 2014a). *SRRM4* was recently shown to regulate the splicing of microexons in neurons (Irimia et al., 2014), which is disrupted in ASD (Irimia et al., 2014) and *srrm4* loss of function in mice leads to neurodevelopmental abnormalities (Quesnel-Vallières et al., 2015).

As a proof of principle, we focused on a single gene, *TRMT1*, which has been implicated in intellectual disability (ID) and whose sSNP falls within a *SRRM4* cross-linking immunoprecipitation sequencing (CLIP-seq) peak (Yang et al., 2015). The strong sQTL signal in *TRMT1* within this CLIP-seq peak (Figure 3G) suggested that *SRRM4* would regulate this intron. Indeed, *SRRM4* overexpression leads to significant differential splicing of the same intron for *TRMT1* in human cells *in vitro* (Parikshak et al., 2016; Raj et al., 2014). Thus, we predict that splicing factors exhibiting significant enrichment with sSNPs provide functional links between allelic variation and the factors that modulate alternative splicing. The subsequent changes in expressed protein sequence are likely to have consequences for downstream neuronal functioning and provide high priority candidates for subsequent mechanistic investigation.

eQTL and sQTL Can Overlap but Are Mostly Independent

We next analyzed the overlap of genes harboring a significant eQTL compared to those with sQTL. Half of all genes with a sQTL were also an eGene (Figure 3I; STAR Methods), and 67% of sQTLs also affect total levels of expression. However, only 22% of eQTLs affect splice-junction usage (STAR Methods), suggesting independent regulation of most expression and splicing events, parallel with observations in other tissues (Li et al., 2016). Most of the identified regions for expression and splicing of the same gene are distinct, as evidenced by the large distances between eSNP and sSNPs influencing the same gene (Figure 3J), with 30% of the genes exhibiting a $D' < 0.7$ between the eSNP and sSNP (Figure S5F). For validation, we also used Ensembl's Variant Effect Predictor (VEP) (McLaren et al., 2016) that annotates SNP function, and observed more sSNPs identified as

an intronic variant than eSNPs and more eSNPs being identified as upstream gene variants (Figure 3K).

Tissue Specificity Corresponds to eQTL Effect Size

To examine eQTL sharing among tissue types, we next examined the correlation of effect sizes of prenatal brain eQTLs to those from 48 different tissue types from the Genotype-Tissue Expression Consortium (GTEx) (STAR Methods) (GTEx Consortium et al., 2017). Prenatal brain eQTLs found in one or more tissues showed significantly lower effect sizes ($p < 2.2 \times 10^{-16}$) than those that were prenatal brain-specific, consistent with previous studies that show eQTLs observed across tissues are more constrained, whereas tissue-specific eQTLs have a greater magnitude of effect (Mohammadi et al., 2017; GTEx Consortium et al., 2017). Prenatal brain-specific eGenes show lower tolerance to loss-of-function mutations as measured by pLI (Lek et al., 2016), compared with those eGenes shared between one or more tissue types (Figure 4B). This contrast between regulatory and coding variation highlights a model whereby tissue specific regulatory control provides a subtler means for evolution to vary protein expression levels, versus the often larger and disruptive effects of protein coding variation. To assess prenatal eQTL sharing between CNS and non-CNS tissues, we correlated the effect sizes of all significant prenatal eQTLs to the same eQTL across all GTEx tissues and PsychENCODE prefrontal cortex (STAR Methods). We observed the strongest correlations of prenatal brain eQTLs with adult brain and proliferative epithelial containing tissues (Figure 4C).

Comparison of Prenatal and Adult eQTL, which Differentially Enrich for SCZ Risk

Another important question is related to developmental stage: how do prenatal brain eQTLs compare with adult brain eQTLs? We first compared eGenes in our dataset to that of GTEx adult cortex (GTEx Consortium et al., 2017). We found 2,532 eGenes that overlapped between prenatal and adult stages, accounting for slightly more than one-third of both datasets (Figure 4D) and 68% of overlapping eQTLs tag the same regulatory region, even if the top SNP differed (Figure S6E; STAR Methods). Additionally, we compared our eGenes to those from PsychENCODE, the largest adult brain dataset to date (Wang et al., 2018a). Although most of the eGenes overlap in this comparison (STAR Methods), we still find more than 1,000 eGenes specific to the prenatal dataset (Figure 4E), which is likely an underestimate of the true number of prenatal specific eQTL, given the prenatal dataset's nearly 10-fold smaller sample size.

We also compared genes that harbor a sQTL in our dataset to sQTLs identified in adult prefrontal cortex (Takata et al., 2017). We found that only 24% of the sGenes identified in prenatal brain were sGenes in the adult brain, which accounts for 39% of the adult brain sGenes (Figure 4F; STAR Methods), suggesting many splicing events may be stage-specific. Because the methods used to detect splicing differ between the studies, this comparison is not ideal, emphasizing the need for more comparable splicing comparisons at different stages of development.

To further examine the differences in eGenes at the different developmental time points, eGenes were annotated based on prenatal cell type markers (STAR Methods) (Polioudakis et

al., 2019). We found that many more prenatal specific eGenes are prenatal cell type markers, compared with GTEx eGenes that are adult-specific (Figure 4G; STAR Methods), consistent with expectations based on the cellular composition of each epoch. One interesting exception are markers for microglia, which is a neural-immune cell present in prenatal and adult brain, but that showed enrichment for prenatal eGenes.

Previous work based on chromatin accessibility during cortical development had suggested that genetic liability for SCZ was significantly enriched in regions active in prenatal brain (de la Torre-Ubieta et al., 2018). Given the many differences between prenatal and adult brain eQTLs at both the level of SNPs and genes, we reasoned that partitioning disease risk imparted by common genetic variation could inform the question as to the timing of genetic contributions to disease risk. We found that SNP-based heritability for SCZ shows prenatal brain eQTLs, followed by sQTLs to be the highest enriched among all significant categories, with eQTLs accounting for 3.7% of SNP heritability ($p = 9.1 \times 10^{-4}$) (Figure 4H) and sQTLs accounting for 2.1% of SNP heritability ($p = 6.6 \times 10^{-3}$). In contrast, adult brain eQTLs from both GTEx and PsychENCODE do not reach significance (Figure 4H), suggesting that prenatal brain enriched regulatory regions harbor a greater proportion of SCZ risk variant than those enriched in adult. However, when the prenatal and adult annotations are combined, the proportion of SNP heritability explained for SCZ increases in an additive manner; the combined set of prenatal eQTLs and PsychENCODE eQTLs explain 6.6% SNP heritability for SCZ. This further supports the other analyses indicating that prenatal and adult regulatory regions are distinct and complementary.

In contrast to adult eQTL, adult sQTLs do contribute significant SNP heritability in SCZ, explaining 2.3% in SCZ, and when combined with the prenatal sQTLs, explain a total of 3.5% of the SNP-based heritability (Figure 4H). To test the robustness of this finding, we explored different window sizes around each eGene (STAR Methods). We observed consistent significant enrichment over almost an order of magnitude window sizes for prenatal annotations over adult for SCZ GWAS loci (Figure 4I).

eQTL within the Context of Transcriptional Networks

We applied robust weighted gene co-expression network analysis (WGCNA) (Zhang and Horvath, 2005) to construct transcriptional networks, identifying 19 modules (labeled by color) of co-expressed genes during mid-gestation cortical development (Figure 5A; Table S3). The modules identified represent genes that correspond to distinct biological functions defined through shared Gene Ontology (GO) enrichments and cell type markers (Figures 5B and 5C; STAR Methods). Six of these modules are enriched for specific brain cell types or brain-relevant GO terms: turquoise (mitotic progenitors and cell division), red (mitotic progenitors, outer radial glia, and splicing), yellow (superficial layer neurons and splicing), blue (developing neurons and axon guidance), green-yellow (adult neurons, synaptic transmission, and neuron projection development), and brown (adult neurons and CA^{2+} transport). These modules, by containing a full range of the major cell types in developing human brain, capture a substantial portion of the biological processes occurring during prenatal cerebral cortical development (Polioudakis et al., 2019; Pollen et al., 2015).

We next leveraged the identified eQTLs to link noncoding variants with target genes and asked whether there were any modules containing genes whose regulatory regions were enriched for ASD and SCZ GWAS signal (STAR Methods). We identified significant enrichments for SCZ (blue; $p = 0.00099$) and a marginal trend toward enrichment for ASD (yellow; $p = 0.061$)-associated common variants (Figures 5G, 5H, and S7). Interestingly, the blue module (Figure 5D), which showed eQTL GWAS enrichment for SCZ, is enriched for the GO category neurogenesis, including genes known to play major roles in brain development, such as *DLX1*, *FGF2*, and *LHX6*. The yellow module (Figure 5F), which shows suggestive eQTL GWAS enrichment for ASD, is enriched for the biological process of gene regulation in developing neurons and includes key genes such as *MEF2*, *HNRNPH3*, and *FOXP4*. Remarkably, single-cell sequencing data indicates that the genes within this module are also enriched in upper layer cortical projection neurons (Polioudakis et al., 2019), consistent with previous data suggesting that ASD-associated variation was enriched in superficial cortical layers (Parikshak et al., 2013). This analysis demonstrates the power of eQTLs, when integrated with co-expression modules to define where common genetic variation associated with a disease acts through regulation of genes with similar biological functions and potentially similar regulatory control.

Next, to determine whether there was overlap in pathways affected by common and rare variation, we examined if genes harboring rare mutations associated with risk for neuropsychiatric disease showed similar enrichment (Figure 5I). We find only the red module enriched for rare variation for SCZ (nominal $p = 0.037$), which is also trending toward enrichment in common variation from SCZ GWAS (Figures 5E and 5G). The yellow module exhibits the most significant enrichment for rare variation in ASD (FDR = 0.011) and is also the most enriched in common variation from ASD GWAS (Figure 5H). These analyses indicate that ASD and SCZ risk variants effect divergent gene sets during brain development. However, they also suggest that the pathways impacted by common and rare genetic variation may converge in each disorder.

eQTL and sQTL Differentially Enrich for GWAS Signal

Recent studies have shown heritability for complex disorders is disproportionately enriched in functional categories such as conserved regions and enhancers (Finucane et al., 2015), as well as regions regulating splicing (Li et al., 2016). However, little is known about how allelic variation affects splicing variation and disease risk in developing human brain, and how this compares to expression, so we compared GWAS enrichment patterns in sQTL and eQTL (Figure 5J; STAR Methods). SCZ (Pardiñas et al., 2018)-associated variants are significantly enriched for both prenatal eQTL and sQTL regions ($p = 0.0004$ and $p = 0.002$, respectively), whereas educational attainment (Okbay et al., 2016) is only significant for sQTL regions and not eQTL ($p = 0.004$) (Figure 5J). Genetic variants associated with risk for attention deficit hyperactivity disorder (ADHD) (Demontis et al., 2019) show a trend for enrichment in both eQTL and sQTL regions, whereas ASD GWAS (Grove et al., 2019) only shows a trend for enrichment in sQTLs (Figure 5J). As a negative control, we tested variants associated with risk for inflammatory bowel disease (Jostinset al., 2012) and observed no enrichment. Overall, these data are consistent with previous suggestions that sQTL harbor substantial disease risk, perhaps even more so than eQTL (Li et al., 2016), highlighting

the relevance of these data to functional characterization of disease-associated risk variants, which will improve as sample sizes increase.

SCZ TWAS Prioritizes Dozens of Novel Risk Genes

To further leverage these data to characterize SCZ loci with developmental origins, we used a transcription-wide association study (TWAS) (Gusev et al., 2016), to integrate *cis*-eQTLs and GWAS to identify genes whose expression is correlated with SCZ. Previous TWAS studies in SCZ both relied on adult brain or non-brain tissues (Gandal et al., 2018b; Gusev et al., 2018). Given the evidence for a neurodevelopmental origin of SCZ (de la Torre-Ubieta et al., 2018; Gulsuner et al., 2013), we reasoned that these prenatal data would provide new perspectives on SCZ risk, especially given the sensitivity of TWAS to tissue source (Wainberg et al., 2017).

We used SCZ GWAS summary statistics (Pardiñas et al., 2018; Ripke et al., 2014) and our prenatal brain eQTL dataset to identify genes and splicing-events whose imputed *cis*-regulated expression is associated with SCZ. We identified 62 genes and 91 introns with significant transcriptome-wide SCZ associations ($P_{\text{Bonferroni}} < 0.05$) (Figure 6A; Table S4; STAR Methods). We also conducted a summary-data-based Mendelian randomization (SMR) and the associated heterogeneity in dependent instruments (HEIDI) test (Zhu et al., 2016) to test for pleiotropic association in the *cis* window of eQTL associations to distinguish pleiotropy from linkage. Eight genes and six introns are significant across both methods ($P_{\text{SMR}} < 0.05$ and $P_{\text{HEIDI}} < 0.05$; Table S5). With support from two different methods, these genes and introns provide high priority targets for further investigation.

We next compared the overlap in SCZ candidate risk genes implicated by either prenatal or adult brain TWAS. Of 60 high-confidence adult brain SCZ TWAS implicated genes (Figure 6B; Table S4; STAR Methods), eleven overlapped with prenatal gene (binomial test $p < 2.2 \times 10^{-16}$; *SNX19*, *VSP29*, *XRCC3*, *TSNAXIP1*, *DNAJA3*, *INO8OE*, *NAGA*, *SF3B1*, *TYW5*, *C2orf47*, and *DDHD2*). These TWAS associations, significant across the multiple adult brain datasets and prenatal brain, implicate genes that are expressed throughout development and may impart risk across developmental stages.

We highlight three of these genes: IN080 complex subunit E (*INO8OE*), splicing factor 3b subunit 1 (*SF3B1*), and matrix AAA peptidase interacting protein 1 (*MAIP1*; *C2orf47*). *INO8OE* is a component of a chromatin remodeling complex involved nucleosome spacing and modulates transcriptional regulation during corticogenesis (Ayala et al., 2018; Sokpor et al., 2018). *SF3B1* is within a SCZ GWAS locus and is supported by an animal model of psychosis (Ingason et al., 2015). *MAIP1* (*C2orf47*) plays a role in mitochondrial Ca^{2+} handling and cell survival and was previously associated with SCZ in a large Swedish GWAS (Ripke et al., 2013). As a comparison, we performed a TWAS incorporating GTEx whole blood expression weights with the SCZ GWAS. We find that only 4 genes overlap between prenatal brain, high confidence adult brain, and whole blood SCZ transcription-level associations (binomial test $p = 2.548 \times 10^{-14}$) (Figure 6C).

At the level of splicing, we identify 13 genes with intron associations that overlapped between the 91 prenatal intron associations and the 120 adult intron associations (Figure

6D). We highlight two genes whose splicing is implicated by both prenatal and adult brain: adaptor related protein complex 3 subunit beta 2 (*AP3B2*) and M-phase phosphoprotein 9 (*MPHOSPH9*). *AP3B2* has been linked to developmental disorders (Assoum et al., 2016) and is a component of the neuron-specific AP-3 complex that has been shown to interact with dysbindin-1, a SCZ-related protein, through direct binding to the AP-3 complex through *AP3B2* (Hashimoto et al., 2009; Oyama et al., 2009). *MPHOSPH9* has been associated with SCZ through differential expression at the exon level in brain, associating splicing abnormalities of *MPHOSPH9* with SCZ (Cohen et al., 2012).

To identify new risk regions for SCZ not identified by GWAS alone, we next examined the overlap between the 145-independent genome-wide significant SCZ GWAS loci (Pardiñas et al., 2018) and significant prenatal brain TWAS associations. We find 50 GWAS loci harbor prenatal TWAS associations and 23 additional SCZ risk loci where significant TWAS loci do not overlap GWAS significant loci (STAR Methods). Of these 23 newly identified loci, 18 regions were also identified by a high confident adult brain TWAS association and 5 were unique to prenatal brain (Table S6; STAR Methods).

We next conducted fine-mapping to refine TWAS associations by accounting for the correlation of linkage disequilibrium (LD) and SNP weights (Mancuso et al., 2019). We identified a credible set of 155 genes, with an average of 3 genes fine-mapped at each GWAS locus (Table S7), largely reflecting local patterns of LD, and indicative of the need for further experimental dissection at many of these complex loci.

A salient example of prenatal and adult TWAS overlap within a known GWAS region is at 16p11.2. This region is complex, containing 6 prenatal associations and 4 adult associations, with only one gene in common to both stages, *INO80E* (Figure 6E). *INO80E* shows both significant co-localization of its prenatal brain eQTL and SCZ GWAS in this region by SMR ($P_{\text{SRM}} \text{INO80E} = 3.7 \times 10^{-6}$) (Figure 6F) and is identified as a casual gene by fine-mapping (Table S7; STAR Methods), making it a high confidence locus identified in both tissues by both methods. *TMEM219* is the only other gene in the credible set fine-mapped to this region and is specific to the prenatal period. Another SCZ GWAS locus on chromosome 3 harbors complex stage-specific splicing and expression associations (Figure 6G). Co-localization of prenatal brain eQTLs by SMR supports *NT5DC2*, *GLN3*, and *PBRM1* at this locus ($P_{\text{SRM}} \text{NT5DC2} = 1.4 \times 10^{-6}$, $P_{\text{SRM}} \text{GLN3 intron} = 2.9 \times 10^{-6}$, $P_{\text{SRM}} \text{PBRM1 intron} = 3.0 \times 10^{-6}$) (Figure 6H), providing multiple lines of evidence linking expression changes in *NT5DC2*, and splicing of *GLN3* and *PBRM1* to SCZ risk. These analyses provide evidence across development supporting *INO80E*, *GLN3*, and *PBRM1* and prenatal specific associations, such as *TMEM219* and *NT5DC2*.

Intracranial Volume TWAS

Given that cortical neurogenesis is a major driver of brain evolution and brain size (Baet et al., 2015; Geschwind and Rakic, 2013; Kostovi and Jovanov-Milosevi, 2006; Rakic, 1995, 2009), we reasoned that these developing cortex eQTL and sQTL would be particularly valuable in defining loci involved in intracranial volume (ICV), which is correlated with brain size in humans (Sgouros et al., 1999). We leveraged GWAS of intracranial volume (Adams et al., 2016) to perform TWAS, identifying 7 genes whose expression

(*NSF*, *LRRC37A*, *LRRC37A2*, *LRRC37A17P*, *RNF123*, *RP11-156P1.3*, *MAPT-AS1*) and 8 introns whose splicing (*NT5C2*, *CRHR1*, *LRRC37A*, *LRRC37A2*, *USMG5*, *KANSL1*, *USP4*) were significantly associated with ICV ($P_{\text{Bonferroni}} < 0.05$) (Figure 7A; Table S4; STAR Methods). We also performed TWAS using adult brain (STAR Methods). *LRRC37A2* is the only gene that overlaps between the prenatal implicated genes and the 16 significant adult cortex genes (Figure 7B; Table S4). Three genes (*CRHR1*, *LRRC37A2*, and *USMG5*) with splicing associations overlapped between the 8 prenatal intron associations and 10 adult intron associations (Figure 7C).

We compared the overlap of significant GWAS regions with significant TWAS associations to identify any new regions harboring genetic modifiers of ICV (STAR Methods). Two prenatal associations (*RNF123* and *USP4*) lie within close proximity to each other on chromosome 3, revealing a new locus associated with ICV. Of the adult loci identified, *GPX1* overlaps the new locus identified from the prenatal brain dataset on chromosome 3, while *IGFBP2* and *CTD-2292M16.8* reveal adult-specific regions on chromosomes 2 and 14, respectively (Table S6).

One particularly interesting GWAS region lies at 17q21, where several significant TWAS associations are found (6 prenatal brain genes, 3 prenatal brain introns, 13 adult brain genes, and 6 adult brain introns) (Figure 7D). This region contains a common inversion polymorphism and has been associated with several brain-related diseases, including SCZ, dyslexia, and Parkinson's disease (Chen et al., 2016; Latourelle et al., 2012; Veerappa et al., 2014). Fine-mapping of the TWAS associations at this region prioritizes *LRRC37A*, which is the only gene significant at both developmental time points (Figure 7E; STAR Methods) (BrainSpan, 2013). The only other ICV GWAS locus overlapping a prenatal brain TWAS association is on chromosome 10 (Figure 7F), which contains significant splicing associations in *NT5C2* and *USMG5*, the latter also harboring an adult splicing association. *USMG5* (*ATP5MD*) is a subunit of the mitochondrial ATP synthase (Ohsakaya et al., 2011), in which mutations cause Leigh syndrome (Barca et al., 2018). *NT5C2* is a hydrolase that plays a role in cellular purine metabolism that has been linked to ID and spastic paraplegia (Darvish et al., 2017) and shows greater prenatal than postnatal expression (Figure 7G) (Brain-Span, 2013).

DISCUSSION

Our analysis provides the largest genome-wide map of human eQTL and the first map of sQTL during cerebral corticogenesis, a critical epoch in brain development, significantly expanding our understanding of gene and splicing regulation in the developing brain. We have comprehensively described the implicated regulatory regions defined by eSNPs and sSNPs, enabling the integration of developmental diversity to previous adult brain functional genomic analysis and genetic variant interpretation. This allows us to identify known major regulators of both expression (TFs) and splicing (RBPs) whose activity is predicted to be affected by common allelic variation. Several of these regulators are known to be disturbed in neurodevelopmental disease via rare variation (Carvill et al., 2013; Irimia et al., 2014; Lai et al., 2001; Suls et al., 2013; Zhang et al., 2014a), providing a link between the activities of common and rare variation on disease risk during brain development. We also

show that genetic variation controlling regulation of expression and splicing in the human brain is sensitive to developmental stage. In the context of early onset neurological and psychiatric disorders, this provides a new window into genetic control of gene expression and splicing regulation during a critical time point for disease development. These data are available in the supplemental tables (Tables S1 and S2), while individual-level raw data has been deposited in dbGaP. To enable easier access to these prenatal brain eQTLs and sQTL, we have built the DevEloPing Cortex Transcriptome (DEPICT) viewer (<https://labs.dgsom.ucla.edu/geschwind/pages/eqtl-browser>), a web portal that allows browsing by gene or SNP summary-level data.

By way of comparison, a smaller prenatal brain eQTL dataset consisting of 120 different samples published while this work was under submission (O'Brien et al., 2018), shows high concordance among significant eQTLs found in both studies (effect size correlation, $r = 0.67$) (Figure S6F; STAR Methods). However, given our sample size, we identified nearly five times the number of eQTLs as that study (6,546 versus 1,329), consistent with the well-described relationship between eQTL discovery and sample size (Nica and Dermitzakis, 2013). Additionally, both studies identify a common inversion polymorphism at 17q21 (Stefansson et al., 2005) which harbors many eGenes. O'Brien et al. (2018) find associations between 7 eGenes in this region and variants associated with neuroticism, including *LRRC37A*, a gene we identify as a prenatal specific TWAS hit for ICV and have fine-mapped to this locus. Together, our studies emphasize the importance of understanding transcriptional regulation during prenatal periods.

We find that integrating eQTLs with WGCNA-defined modules from prenatal brain expression shows distinct GWAS enrichment for ASD and SCZ within specific biological processes indicating disease-specific biology. This substantially advances previous work based on rare, gene disrupting *de novo* mutations (Iossifov et al., 2012; Ruzzo et al., 2019; Sanders et al., 2012) by showing convergence in genetic risk factors, even at the level of common variants that lie in regulatory regions. We show that genes involved in chromatin organization and splicing, as well as cell-type markers for superficial layer neurons are enriched for ASD GWAS loci in their eQTL-defined regulatory regions, similar to rare variants. For SCZ, we find enrichment of the biological pathways of neurogenesis and CNS development, parallel to previous observations based on analysis of chromatin confirmation in prenatal brain (Won et al., 2016), providing independent support for neurogenesis as a key process disrupted in SCZ risk. Furthermore, we show that SCZ GWAS enrichment from partitioned heritability significantly differs when using prenatal eQTL annotations versus adult eQTL annotations, consistently indicating the prenatal annotations to be the highest enriched among all functional annotations even when comparing to the PsychENCODE dataset with a sample size nearly 10 times larger than this cohort. Furthermore, the heritability explained by prenatal and adult eQTLs shows an additive effect, indicating the regulatory regions identified at these distinct time points indeed differ and are capturing different aspects of disease risk.

Similar significant enrichment of SCZ partitioned heritability has been shown for prenatal brain assay for transposase-accessible chromatin using sequencing (ATAC-seq) peaks (de la Torre-Ubieta et al., 2018), providing multiple lines of evidence that regulatory regions active

in early cortical development harbor significant SCZ risk and are likely to be a crucial site of action for disease risk. Additionally, genetic risk for ADHD implicates prenatal eQTL and sQTL, while ASD risk shows a trending enrichment in sQTL. These trending enrichments are likely to gain significance as GWAS sample sizes increase. The identification of sQTL from prenatal brain will further allow mechanistic exploration of the effects of disease risk on gene isoform expression. Particularly interesting in this regard is the set of known splicing factors whose targets are enriched for disease-associated variation.

Finally, by integrating our map of prenatal gene expression and splicing regulation with the SCZ and ICV GWAS via TWAS, we are able to identify new candidate genes and candidate molecular mechanisms through which these disease-associated variants may be acting. Moreover, when comparing the implicated genes from prenatal brain expression with genes implicated via adult brain expression, there is little overlap. To some degree, this is expected and reflects TWAS's known sensitivity to tissue (Wainberg et al., 2017), as well as the differences in eGenes and effect sizes between prenatal and adult eQTLs shown in our analysis. It does, however, highlight the importance of picking appropriate tissues and gene expression studies for a given phenotype. Here, the SCZ risk genes predicted by TWAS based on prenatal brain expression contributes to the growing list of candidate genes discovered from adult expression for a more complete view of potentially causal genes and splicing events. These results and others (de la Torre-Ubieta et al., 2018; Won et al., 2016) are consistent with the original framing of SCZ as a neurodevelopmental disorder (Weinberger, 1987). These data indicate that the developmental sensitivity for SCZ lies not only in adolescent development, but in the earliest periods of cortical neurogenesis and cortical patterning. This epoch is also particularly important for brain volume and patterning (de la Torre-Ubieta et al., 2018; Geschwind and Rakic, 2013), and the integration of eQTL data with the ICV GWAS identifies many new genes influencing human brain size.

GWAS regions that yield discordant TWAS gene associations depending on the developmental time period of reference panel are also interesting to consider. A salient example is the region containing the 16p11.2 copy number variant (CNV), which has been associated with multiple neurodevelopmental conditions, including SCZ, ASD, ID, and bipolar disorder (Bernier et al., 2017; Hanson et al., 2015; Shinawi et al., 2010; Weiss et al., 2008; Zhou et al., 2018). Fine mapping of this locus in SCZ implicates *INO8OE* and *TMEM219* from prenatal brain, *INO8OE* being the only gene in this locus implicated by both prenatal and adult data. *INO8OE* is an interesting candidate, due to its similar role as the chromatin-helicase-DNA binding protein family, including *CHD8*, in chromatin remodeling during cortical neurogenesis (Clapier et al., 2017; Sokpor et al., 2018). Prenatal brain TWAS also implicated *KCTD13*, *CTD-2574D22.2*, *PPP4C*, and *YPEL3*, whereas *MAPK3*, *DOC2A*, and *TAOK2*, although implicated by adult brain expression data, are not supported by fine-mapping. This CNV contains 29 genes, so the relationship of this CNV to phenotype is unlikely to be simple or be driven by only one gene (Escamilla et al., 2017; Golzio et al., 2012). Additional fine mapping and functional analyses will be necessary for more conclusive genotype-phenotype associations.

These results demonstrate the importance of considering developmental stage of brain expression when using eQTL and sQTL data to interpret disease associated variants. They

also highlight the value of using these data to further characterize developmental time points during which genetic variation acts to modulate risk for neurodevelopmental and early onset neuro-psychiatric diseases, suggesting that more detailed maps of the effects of common genetic variation effects throughout the lifespan will be of value.

STAR★METHODS

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Daniel H. Geschwind (dhg@mednet.ucla.edu). Summary statistics are available in the supplemental tables (Tables S1 and S2), while individual-level raw RNA-seq and genotype data has been deposited in dbGaP: phs001900. Also, to enable easier access to these prenatal brain eQTLs and sQTL, we have built the DevEloPing Cortex Transcriptome (DEPICT) viewer (<https://labs.dgsom.ucla.edu/geschwind/pages/eqtl-browser>), a web portal that allows browsing by gene or SNP summary-level data.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Developing Human Brain Samples—Prenatal tissue was obtained from the UCLA Gene and Cell Therapy core according to IRB guidelines from 233 donors (post-conception weeks: 14–21, final sample of 201 = 84 Female, 117 Male) following voluntary termination of pregnancy. This study was performed under the auspices of the UCLA Office of Human Research Protection, which determined that it was exempt because samples are anonymous pathological specimens. Full informed consent was obtained from all of the parent donors.

METHOD DETAILS

Library Preparation—Total RNA and genomic DNA from human prenatal brain tissue from PCW 14–21 that visually appeared to be cortical was extracted using miRNeasy-mini (QIAGEN) and DNeasy Blood and Tissue Kit (DNA) or were extracted using trizol with glycogen followed by column purification. Library preparation via Illumina Stranded TruSeq kit with Ribozero Gold ribosomal RNA depletion library prep was followed by sequencing on 233 brains and genotype array data on 212 brains was generated at the UCLA Neurogenomics Core. Pseudo-randomization was performed to decrease correlation between sequencing lane and biological variables such as sex and gestation week. RNA samples were pooled, randomized, and run across 4 lanes. Ribozero, ribosome depleted, 50 bp paired-end RNA sequencing was performed with mean sequencing depth of 60 million reads on an Illumina HiSeq2500.

QUANTIFICATION AND STATISTICAL ANALYSIS

Genotype pre-processing—Genotyping was performed at the UCLA Neurogenomics Core (UNGC) on either Illumina HumanOmni2.5 or HumanOmni2.5Exome platform in 8 batches. SNP genotypes were exported into PLINK format. Batches were merged and markers that did not overlap genotyping platforms were removed. SNP marker names were converted from Illumina KGP IDs to rsIDs using the conversion file provided by Illumina. Quality control was performed in PLINK v1.9 (Chang et al., 2015). SNPs were filtered based on Hardy-Weinberg equilibrium ($-hwe\ 1e6$), minor allele frequency ($-maf\ 0.01$),

individual missing genotype rate (-mind 0.10), variant missing genotype rate (-geno 0.05) resulting in 1,799,583 variants (Figure S1).

RNA-sequencing Data Processing Pipeline—All raw RNaseq fastq files, 4 per sample run on different lanes, were run through FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). FastQC output was visually inspected and sequencing lanes where the “per tile sequence quality” was red were removed, there was no sample with more than one sequencing lane removed. Fastq files were aligned to the GRCh37.p13 (hg19; Homo_sapiens.GRCh37.75.dna.primary_assembly.fa: ftp://ftp.ensembl.org/pub/release-75/fasta/homo_sapiens/dna/) reference genome using STAR v2.4 (Dobin et al., 2013). SAM files were sorted, indexed, converted to BAM files and merged across lanes from the same sample using Samtools v1.2 (Li et al., 2009). Gene quantifications were calculated using HTSeq-counts v0.6.0 (Anders et al., 2015) using an exon union model on the basis of Gencode v19 comprehensive gene annotations (Harrow et al., 2012). Quality control metrics were calculated using PicardTools v1.139 (<http://broadinstitute.github.io/picard>) and Samtools. A sex incompatibility check was also performed using XIST expression and Y chromosome non-pseudoautosomal expression which are known to show different patterns of expression in males and females. A scatterplot of XIST expression versus the sum of expression of genes in the non-pseudoautosomal region of the Y chromosome showed no gender mismatches. (Figure S1)

Sample Swap Identification—QC'd genotypes and sample BAM files were used to identify any sample identity swaps between the RNA and DNA experiments using VerifyBamID v1.1.2 (Jun et al., 2012). We identified 4 samples where [CHIPMIX] ~1 AND [FREEMIX] ~0, indicative of unmatching RNA and DNA, which were removed in the VCF file.

Genotyping Pipeline—PLINK genotype files were converted to *vcf* files using Plinkseq v0.10 (<https://atgu.mgh.harvard.edu/plinkseq/>). Genotypes were imputed into the 1000 Genomes Project phase 3 multi-ethnic reference panel (1000 Genomes Project Consortium et al., 2015) by chromosome using Beagle v4.1 (Browning and Browning, 2016) and subsequently merged. Multiallelic sites were removed using GATK v3.5 (Van der Auwera et al., 2013). Imputed genotypes were filtered for Hardy-Weinberg equilibrium p value $< 1 \times 10^{-6}$ and minor allele frequency (MAF) 5%. Imputation quality was assessed filtering variants where allelic R-squared > 0.5 and dosage R-squared > 0.5 by GATK, resulting in ~6.6 million autosomal SNPs. We restricted to only autosomal due to sex chromosome dosage, as commonly done (GTEx Consortium, 2015).

RNA-seq Quality Control and Normalization—Gene counts were compiled from HTSeq Count (Anders et al., 2015) quantifications and imported into R version 3.2.1 for downstream analyses. Gene counts were put through quality control, removing genes that were not expressed in 80% of samples with 10 counts or more. Expression was then corrected for GC content, gene length, and quantile normalized to a standard normal distribution, as commonly done in QTL studies (Battle et al., 2015). Sample outliers were removed based on standardized sample network connectivity Z scores < 2 (Zhang and

Horvath, 2005) Figure S2F). ComBat batch correction was performed (Johnson et al., 2007). After quality control and normalization, there remained 201 samples with 15,930 genes expressed (on the basis of Gencode v19 annotations) at sufficient levels (gene biotypes include 12,943 protein coding, 767 long noncoding RNAs, among others).

Covariate Selection—To evaluate and remove global effects of gene expression, we used PLINKv1.9 (Chang et al., 2015) to run multidimensional scaling on the QC'd imputed genotypes and to verify ancestral backgrounds of the samples. We aggregated the final 201 samples with HapMap3 (International HapMap Consortium, 2003) of 1397 samples across 11 populations (87 ASW, 165 CEU, 137 CHB, 109 CHD, 101 GIH, 113 JPT, 110 LWK, 86 MXL, 184 MKK, TSI 102, YRI 203). A plot of the first two MDs components of the merged data shows the genetic ancestry of our samples among a diverse reference population (Figure S2H). For eQTL analysis, the top 3 MDS components calculated only in the prenatal brain samples were used as covariates.

We also looked at the correlation of known measured biological covariates, measured technical covariates, as well as RNA quality control metrics from Picard tools (gestation week, RIN, sex, purification method, 260:230 ratio, 260:280 ratio, read depth, percent chimeras, 5' bias, 3' bias, AT dropout) with the top 10 principle components of the expression data and find the top principal component corresponds to the age of the sample (gestation week) and the second component corresponds to the RNA integrity number (RIN) (Figure S2G).

To measure hidden batch effects and confounders, hidden covariate analysis was performed using Hidden Covariates with a Prior (HCP) (Mostafavi et al., 2013). Hidden factors were calculated given the known measured factors. HCP was run separately for varying number of inferred hidden components: 5,10,15,20,25, 30. We included 20 HCPs in our eQTL model which we found to maximized eGene discovery along with gestation week, RIN, and sex (Figures S3A and S3C). We correlated the 20 HCPs along with gestation week, RIN, sex, and top 3 genotype PCs (all covariates used in the final model) with the measured factors and Picard metrics, as well as the top 20 PCs of expression to gain insight to meaning of the HCPs. We see each inferred hidden component's relationship to the known variables is complex and distributed across variables.

cis-eQTL mapping—We performed cis-eQTL mapping using FastQTL (Ongen et al., 2016), a defined *cis* window of 1 megabase up- and down-stream of the gene start site regardless of strand for 15,930 expressed genes, and correction for the following covariates: gestation week, RIN, sex, 20 HCPs, 3 genotype PCs. FastQTL (Ongen et al., 2016) was run in the permutation pass mode (1000 permutations) to identify the best nominal associated SNP per phenotype and with a beta approximation to model the permutation outcome (Figure S3E) and correct for all SNPs in LD with the top SNP per phenotype. Beta approximated permutation p values were then multiple test corrected using the q-value Storey and Tibshirani FDR correction (Storey and Tibshirani, 2003). We define eQTL containing genes (eGenes) by having an FDR q-value ≤ 0.05 . Secondary, independent eQTLs were identified by rerunning permutation tests in FastQTL for every eGene conditioning on the primary eSNP. To compare with studies that did not perform

a permutation procedure, we tested all SNP-gene pairs and discover 893,813 significant eQTLs (5% FDR) corresponding to 11,625 eGenes.

To assess inflation in a Q-Q plot, we randomly chose 10 genes to run eQTL analysis in *trans*, testing all SNPs genome-wide with association with gene expression using MatrixEQTL (Shabalín, 2012). We corrected for the same covariates as in the cis-eQTL analysis. The Q-Q plot of the trans-eQTLs shows no inflation, an indication that our eQTL results are not confounded by population stratification. (Figure S3F)

qPCR—To validate a few eQTLs, we selected eGenes with the highest effect sizes in order to be able to detect a difference by qPCR and stratify by genotype, in which we find concordant significant eQTL signal. qPCR was performed on 5 samples of genotype 0 and 5 samples of genotype either 1 or 2 for each gene. (Figures S3G–S3I)

EMMAX

To further check that our eQTLs are not due to the population differences of our samples, we ran cis-eQTL analysis with EMMAX (Kang et al., 2008), which accounts for population structure using a genetic relationship matrix. We used the `emmax-kin` function (`-v -h -s -d 10`) to create the IBS kinship matrix. EMMAX was run for each gene with a cis-window of ± 1 MB around the TSS, correcting for the same covariates in the FastQTL analysis. Nominal EMMAX p-values were corrected for multiple testing using the q-value Storey and Tibshirani FDR correction (Storey and Tibshirani, 2003).

To assess overlap between FastQTL and EMMAX, FastQTL was also run in the nominal pass mode to obtain nominal p values for all cis-SNPs tested per gene. FastQTL nominal p values were also corrected for multiple testing using the q-value Storey and Tibshirani FDR correction. We discover 920,356 nominal eQTLs at a 5% FDR threshold. To compare nominal results, we defined eGenes as a gene containing a significant SNP association at FDR ≤ 0.05 . We found 93.8% of eGenes from the FastQTL analysis was an eGene in the EMMAX analysis. Additionally, we compared all SNP-gene pairs tested and found 92.8% of significant SNP-gene associations from nominal FastQTL to be significant in the EMMAX analysis (Figures S4D and S4E).

Meta-Analysis—We split up our sample into six groups based on hierarchical clustering of the top 3 PCs of the genotype data. (Figure S4B) The size of each group ranged from 12 samples to 47 samples and each group corresponded to distinct ancestries based on the MDS plots of samples merged with HapMap3. We performed association testing between the top SNP per gene, identified by FastQTL permutation pass eQTL analysis, within group using the `lm()` function in R, correcting for gestation week, RIN, age, and 20HCPS. A fixed effect meta-analysis was then run between groups using METAL v3.25.2011 (Willer et al., 2010), which implements a Cochran's Q test for heterogeneity. We find significant heterogeneity at 10% of our eQTLs and find 87% of our eQTLs are significant in the meta-analysis at FDR 0.05% (q-value) strongly suggesting our results are not due to population stratification (Figure S4C).

Intron Cluster Quantifications—We used Leafcutter (Li et al., 2018) to leverage information from reads that span introns to quantify clusters of variably spliced introns. From the already aligned FASTQ files by STAR, output bam files were converted into junction files. Intron clustering was performed using default settings of 50 reads per cluster and a maximum intron length of 500kb. The Leafcutter `prepare_genotype_table` script was then used to calculate intron excision ratios and to filter out introns used in less than 40% of individuals with almost no variation. Intron excision ratios were then standardized and quantile normalized. Leafcutter's `leafviz` annotation code were used to annotate detected introns as annotated or cryptic. New cryptic introns were annotated by being cryptic 5', cryptic_3', cryptic unanchored, or novel annotated pair based on Gencode hg19 gene annotations.

sQTL mapping—Standardized and normalized intron excision ratios calculated by leafcutter was used as the phenotype for sQTL mapping. FastQTL (Ongen et al., 2016) was used to test for association between SNPs within a cis-region of ± 100 kb of the intron cluster and intron ratios within cluster. Hidden covariate analysis was performed using Hidden Covariates with a Prior (HCP) (Mostafavi et al., 2013) on intron excision ratios given the same known covariates used for eQTL HCP calculations. We included 5 HCPs in our spliceQTL model which we found to maximized intron QTL (Figure S3D) discovery along with gestation week, RIN, and sex. FastQTL was run in the permutation pass mode (1000 permutations). Beta approximated permutation p values were then multiple test corrected using the q-value Storey and Tibshirani FDR correction. We define sQTL as an intron having an FDR q-value ≤ 0.05 , and an sGene as a gene containing a significant sQTL at any intron.

ATAC-seq Overlap of eQTLs—Prenatal brain ATAC-seq peaks were obtained from (de la Torre-Ubieta et al., 2018). We annotated eQTLs as being supported by ATAC if the LD block ($r^2 > 0.8$ PLINK) around its eSNP overlapped an open chromatin region. To test for significance, we created a null set of eQTLs (q-value > 0.2), annotated overlap with ATAC peaks, and then ran a Fisher's exact test.

Hi-C Overlap of eQTLs—Prenatal brain CP and GZ Hi-C topological association domain bed files were obtained from Won et al. (2016). eSNPs located within 10kb of the eGene TSS were removed, as Hi-C cannot detect any chromosomal interaction less than 10kb apart. We defined any remaining eQTL as overlapping Hi-C if the LD block ($r^2 > 0.8$ PLINK) around its eSNP fell in one 10kbTAD bins and the corresponding eGene ± 2 kb overlapped with the other 10kb TAD bin in either CP or GZ. To test for significance, we created a null set of eQTLs (q-value > 0.2), annotated overlap with Hi-C, and then ran a Fisher's exact test.

Functional enrichment of QTLs in epigenetic marks, transcription factor, and splicing factor binding sites—We performed functional enrichment of both eQTLs and sQTLs using GREGOR (Genomic Regulatory Elements and Gwas Overlap algoRithm) (Schmidt et al., 2015) to evaluate enrichment of variants in genome wide annotations. We downloaded the 25 state ChromHMM model BED files from the Roadmap Epigenetics Project (Ernst and Kellis, 2015; Roadmap Epigenomics Consortium et al., 2015), generated

from a set of 5 core chromatin marks assayed in prenatal brain (Figure S5). We downloaded consensus transcription factor and DNA-binding protein binding site BED files (Arbiza et al., 2013), which called consensus binding sites from multiple cell types from Encode CHIP-seq data which was used to computationally annotate all possible genome-wide sites for 78 binding proteins. We filtered to 62 binding proteins that showed cortical brain expression in BrainSpan (BrainSpan, 2013). Lastly, we obtained human RNA binding protein (RBP) binding site BED files from CLIPdb (Yang et al., 2015) database of publicly available cross-linking immunoprecipitation (CLIP)-seq datasets from 51 RBPs.

GREGOR evaluates the enrichment of QTL variants in these genomic annotations by estimating the significance of observed overlap of the eSNP or sSNP relative to the expected overlap using a set of matched control variants. GREGOR creates a list of possible causal SNPs by extending the list of eSNPs or sSNPs (index SNPs) to all SNPs in high linkage disequilibrium ($r^2 > 0.7$). A set of matched control SNPs (SNPs are selected based on matching the index SNP for number of variants in LD, minor allele frequency, and distance to nearest gene/intron) is then created, and enrichments are calculated based on the observed and expected overlap within each annotation.

We downloaded *CHD8* ChIP-seq peaks from ChIP experiments performed in human mid-gestation prenatal brain (Cotney et al., 2015) and expression counts for *CHD8* knockdown in neural progenitors (Sugathan et al., 2014). ChIP-seq peaks were overlapped with prenatal eQTLs using the GenomicRanges package in R. eGenes were identified in the knockdown and control expression data and plotted using ggplot.

We downloaded *SRRM4* CLIP-seq peaks (Yang et al., 2015) and PSI values from *SRRM4* overexpression in human 293T cells from averaged from 3 overexpression samples and 3 control samples (Parikshaket al., 2016; Raj et al., 2014). CLIP-seq peaks were overlapped with prenatal sQTLs using the GenomicRanges package in R. The leafcutter identified intron of the sGene was identified in the overexpression and control PSI data and plotted using ggplot.

eQTL sQTL Overlap—We used the Storey's π_1 statistic described in Nica et al. (2011), to assess the proportion of true associations among sQTLs that were also detected by the eQTL analysis and eQTLs that were also detected by the sQTL analysis. The overlap was assessed by taking all significant SNP-gene associations from the eQTLs and estimating the proportion of true associations (π_1) on the distribution of corresponding p values of the overlapping SNP-gene pairs in the sQTL dataset and vice versa. This is done by first estimating π_0 , the proportion of true null associations based on their distribution. Then $\pi_1 = 1 - \pi_0$ estimates the lower bound of true positive associations.

Estimation of Variant Effect of eQTL and sQTL—Ensembl's Variant Effect Predictor (VEP) version 90 (McLaren et al., 2016) was used to annotate the effects of variants of significant QTLs on genes, transcripts, protein sequence, and regulatory regions. VEP annotations are based off of a wide range of reference data including Ensembl database version 92, GRCH37.p13 genome assembly, Gencode 19 gene annotations, RefSeq 2015–01,

Poly-Phen 2.2.2, SOFT 5.2.2, dbSNP 150, COSMIC 81, ClinVar 2017–06, and gnomAD r2.0.

Prenatal Cell Type Markers—Cell type enriched genes were obtained from Polioudakis et al. (2019), a single-cell RNA-seq dataset from GW17–18 human prenatal cortex. Briefly, Drop-seq was run on single cells isolated from human prenatal neocortex according to the online Drop-seq protocol v.3.1 (<http://mccarrolllab.com/download/905/>) and the methods published in Macosko et al. (2015). The raw Drop-seq data was processed using the Drop-seq tools v1.12 pipeline from the McCarroll Laboratory (<http://mccarrolllab.com/wp-content/uploads/2016/03/Drop-seqAlignmentCookbookv1.2Jan2016.pdf>). Normalization was performed using Seurat v2.0.1 (Butler et al., 2018). Raw counts were read depth normalized by dividing by the total number of UMIs per cell, then multiplying by 10,000, adding a value of 1, and log transforming ($\ln(\text{transcripts-per-10,000} + 1)$) using the Seurat function ‘CreateSeuratObject’. To identify cell-type enriched genes, differential expression analysis was performed for each cluster individually versus all other cells in the dataset for genes detected in at least 10% of cells in the cluster. Differential expression analysis was performed using a linear model implemented in R as follows: $\text{lm}(\text{expression_number_of_UMI} + \text{donor} + \text{lab_batch})$. P values were then Benjamini-Hochberg corrected (Benjamini and Hochberg, 1995). Genes were considered enriched if they were detected in at least 10% of cells in the cluster, $> 0.2 \log_2$ fold enriched, and Benjamini-Hochberg corrected p value < 0.05 . Cell type enriched genes were annotated based on the gene harboring an eQTL in either the prenatal brain dataset or GTEx adult cortex dataset (GTEx Consortium et al., 2017).

Cross age-tissue comparison—We downloaded GTEx v7 eQTL summary statistics for all 48 tissue types (GTEx Consortium et al., 2017). To compare effect sizes consistently between studies, we calculated effect sizes by running a linear model with scaled log tpm expression values for significant prenatal eQTLs and calculated in the same way for corresponding SNP-gene pairs in the GTEx data to obtain a beta value from non-standard normalized expression. Significant prenatal eQTLs were identified as prenatal specific if the corresponding SNP-gene pair was not found in any GTEx tissue or as shared if it was found in at least one GTEx tissue.

Effect size correlations between all GTEx tissues and PsychENCODE (GTEx Consortium et al., 2017; Wang et al., 2018a) were calculated by first obtaining all FDR ≤ 0.05 (q-value) nominal prenatal eQTLs. Nominal eQTL analysis was run in FastQTL, using the same input for the permutation pass, to obtain all SNP-gene pairs tested. Spearman’s ρ correlations were calculated per tissue on the absolute value of the slope from FastQTL output of all FDR

≤ 0.05 prenatal eQTLs and corresponding absolute value of slope from SNP-gene pairs in GTEx nominal associations. The absolute value of the slope was used for all correlations to control for strandflips. For each tissue compared in this analysis, we indicate how many of the prenatal eGenes were found in the tissue of comparison by the sample size of that tissue (Figure S6C). A scatterplot of the prenatal brain versus PsychEncode adult brain effect sizes shows corresponding eQTL regression beta values from each dataset (Figure S6D).

Additionally, we used Storey's Qvalue software (Storey and Tibshirani, 2003) to assess overlap between prenatal brain eQTLs and the eQTLs from the GTEx v7 tissues (GTEx Consortium et al., 2017). The proportion of true associations (π_1) was estimated by looking up significant prenatal brain eQTLs in each of the GTEx tissues, creating a distribution of corresponding p values of the overlapping SNP-gene pairs used to calculate π_0 , the proportion of true null associations based on their distribution. Then $\pi_1 = 1 - \pi_0$ estimates the lower bound of true positive associations. We performed the reciprocal overlap by looking up GTEx significant eQTLs per tissue in the prenatal brain dataset (Figures S6A and S6B)

Overlap between prenatal brain eGenes and GTEx Cortex eGenes (N = 136 individuals, eGenes = 6,146) was performed by intersecting all significant eGenes detected by permutation test followed by FDR correction. Overlap between prenatal brain eGenes (11,625; 5% FDR all SNP-gene pairs) and PsychENCODE eGenes (N = 1,866, eGenes = 32,944), was performed by intersection all significant eGenes detected by all nominal eQTLs with an FDR = 0.05. Overlap between prenatal brain sGenes and adult sGenes was performed by intersecting the genes of significant sQTLs by permutation test followed by FDR correction.

Overlap between our prenatal brain eQTL dataset and a prenatal brain eQTL dataset consisting of 120 samples (O'Brien et al., 2018), were performed by intersecting all nominal eQTLs with an FDR = 0.05. Spearman's ρ correlations were calculated for eQTL effect sizes (slope) for overlapping FDR-significant eQTLs (Figure S6F).

Partitioned Heritability—Partitioned heritability was measured using LD Score Regression v1.0.0 (Finucane et al., 2015) to identify enrichment of GWAS summary statistics among functional genomic annotations by accounting for LD, specifically eQTL and sQTL regulatory regions. The full baseline model of 53 functional categories was downloaded from Finucane et al. (2015) (<https://github.com/bulik/ldsc/wiki/Partitioned-Heritability>). Prenatal and adult eQTL annotations from both GTEx Cortex and PsychENCODE, and prenatal sQTL and adult sQTL annotations (Takata et al., 2017), were created by taking a 500bp window (+/-250) around each eSNP or sSNP. This resulted in 6,163 prenatal eQTL annotations, 5,690 adult eQTL GTEx annotations, 32,944 adult PsychENCODE eQTL annotations, 4,635 prenatal sQTL annotations, and 8,966 adult CMC sQTL annotations. An annotation file was then created by marking all HapMap3 (International HapMap Consortium, 2003) SNPs that fell within the QTL annotations. LD scores were calculated for the QTL annotation SNPs using an LD window of 1cM using LD reference panel 1000 Genomes European Phase 3 (1000 Genomes Project Consortium et al., 2015). This LD reference panel was chosen due to the CLOZUK+PGC SCZ GWAS (Pardiñas et al., 2018; Ripke et al., 2014) being comprised of mainly European ancestry samples (Ripke et al., 2014). Baseline LD-scores and QTL LD-scores were simultaneously included in computation of partitioned heritability. Enrichment for each annotation was calculated by the proportion of heritability explained by each annotation divided by the proportion on SNPs in the genome falling in that annotation category. Enrichment p values were then Bonferroni corrected. To test robustness of partitioned heritability with 500bp

window annotation, we explored different window sizes (500bp, 1000bp, 5kb, 10kb, 20kb, 50kb, 100kb, 250kb) around each eGene.

All prenatal eQTLs and sQTLs were tested separately for enrichment in genetic variants associated with SCZ (Pardiñas et al., 2018; Ripke et al., 2014), major depressive disorder (CONVERGE Consortium, 2015), intracranial volume (Adams et al., 2016), inflammatory bowel disease (Jostins et al., 2012), head circumference (Taal et al., 2012), epilepsy (International League Against Epilepsy Consortium on Complex Epilepsies, 2014), educational attainment (Rietveld et al., 2013), cognitive performance (Benyamin et al., 2014), ASD (Grove et al., 2019), Alzheimer's disease (Lambert et al., 2013), and attention deficit hyperactivity disorder (Demontis et al., 2019).

WGCNA

After gene counts were put through quality control removing genes that were not expressed at a level of 10 counts or more in 80% of samples, expression was conditional quantile normalized, adjusting for gene length and GC content. Sample outliers were removed based on standardized sample network connectivity Z scores < 2 . ComBat batch correction was performed (Johnson et al., 2007). Gestation week, RIN, and the top 4 Picard PCs were regressed from the expression dataset.

Network analysis was performed with robust consensus WGCNA (rWGCNA) (Zhang and Horvath, 2005) assigning genes to specific modules based on biweight midcorrelations among genes. Soft threshold power of 11 was chosen to achieve scale-free topology ($r^2 > 0.8$) (Figures S7A and S7B). Then, 50 signed co-expression networks were generated on 50 independent bootstraps of the samples; each co-expression network uses the same estimated power parameter. The 50 topological overlap matrices were combined edge-wise by taking the median of each edge across all bootstraps. The topological overlap matrices were clustered hierarchically using average linkage hierarchical clustering (using '1 - TOM' as a dis-similarity measure). The topological overlap dendrogram was used to define modules using minimum module size of 100, deep split of 4, merge threshold of 0.2, and negative pamStage.

Module preservation was run in an independent RNA-seq dataset of cortical development from 8 post conception weeks to 12 months after birth (Parikshak et al., 2013; Sunkin et al., 2013), showing that these modules are all highly preserved (Figure S7).

GO enrichment—GO definitions were downloaded from Ensembl release 86. GO terms with a small (< 35) or large (> 100) number of genes were removed. Logistic regression was performed using the model: $is.go \sim is.module + gene\ covariates$ (GC content and gene length) for an indicator-based enrichment, and p values were Bonferroni FDR corrected. The top two significant terms are reported.

Cell Type enrichment—Cell type markers from human and mouse brain were downloaded from Hawrylycz et al. (2015), Lein et al. (2007), Mancarci et al. (2017), Miller et al. (2014), Tasic et al. (2016), Winden et al. (2009), Zhang et al. (2014b, 2016) as well as the prenatal types from Polioudakis et al. (2019). Logistic regression was performed using

the model: $\text{is.cell type} \sim \text{is.module} + \text{gene covariates}$ (GC content and gene length) for an indicator-based enrichment, and p values were Bonferroni FDR corrected. The top two significant cell types are reported.

Rare variant enrichment—Genes containing disease associated *de novo* variants were downloaded from denovo-db v1.5, which is a collection of germline *de novo* single nucleotide variants and small indels consolidated across many studies for many disorders including for ASD, Developmental Disorder, ID, and SCZ (<http://denovo-db.gs.washington.edu>) De Rubeiset al., 2014; Fromeretal., 2014; Girirajan et al., 2013; Glessner et al., 2009; Gulsuner et al., 2013; International Schizophrenia Consortium, 2008; Iossifov et al., 2014; Krumm et al., 2015; Levinson et al., 2011; Malhotra and Sebat, 2012; Marshall et al., 2008; McCarthy et al., 2009, 2014; Michaelson et al., 2012; Moreno-De-Luca et al., 2010, 2013; O’Roak et al., 2012, 2014; Rujescu et al., 2009; Sanders et al., 2011; Stefansson et al., 2005; Tavassoli et al., 2014; Turner et al., 2016,2017; Vacicetal., 2011; Weiss et al., 2008; Yuen et al., 2015). We compiled a list of all genes containing at least one *de novo* mutation of annotation functional class: frameshift, frameshift near splice, splice acceptor, splice donor, start lost, stop gained, stop gained near splice, and stop lost. We then analyzed the union of these genes with genes implicated by CNVs in Gandal et al. (2018a). Gandal et al. (2018a) included CNVs that were in at least two studies with a p value < 0.01 or in one study with a p value < 10^{-6} , from well powered studies of over 500 subjects per group.(Girirajan et al., 2013; Glessner et al., 2009; International Schizophrenia Consortium, 2008; Levinson et al., 2011; Malhotra and Sebat, 2012; Marshall et al., 2008; McCarthy et al., 2009; Moreno-De-Luca et al., 2010, 2013; Rujescu et al., 2009; Sanders et al., 2011; Stefansson et al., 2005; Vacic et al., 2011; Weiss et al., 2008). Logistic regression was performed using the model: $\text{is.disease} \sim \text{is.module} + \text{gene covariates}$ (GC content and gene length) for an indicator-based enrichment, and p values were Bonferroni FDR corrected.

Disorder	# Genes with <i>de novo</i> variants	# Genes with CNVs	Total # unique genes
ASD	688	288	966
Developmental Disorder	516	0	516
ID	167	0	167
SCZ	81	289	365

GWAS Module Enrichment—To assess module enrichment of GWAS by gene regulatory regions, we first created a map of gene specific regulatory regions. Every eGene was mapped to its eSNP (1st and 2nd if it had one) and extended to the LD block ($r^2 > 0.8$ PLINK) calculated in our sample. GWAS p values for ASD (Grove et al., 2019) and SCZ (Ripke et al., 2014) were assigned to modules based on overlapping positions with eSNP LD block annotations. To assess significance, 1000 permutations were performed for each module, randomly selecting the number of annotations corresponding to the number of genes in each module from all eSNP LD block annotations (Figures S7E–S7G). Significance was calculated by the proportion of permuted observed p values at the expected p value of

0.001 that are larger than the actual module's observed p value at the expected p value of 0.001.

TWAS

We performed SCZ and intercranial volume TWASs using the FUSION package (<http://gusevlab.org/projects/fusion/>) with gene and splice expression measured in prenatal brain tissue. To identify genes with evidence of genetic control, we used GCTA (Yang et al., 2011) software to estimate cis-SNP heritability h^2_g (± 1 MB window around gene TSS). We identified 3,784 genes and 5,738 splicing-events with significant cis- h^2_g (nominal p value < 0.05), which were used to calculate the SNP-based predictive weights per gene/intron.

Using the FUSION package, five-fold cross-validation of five models of expression prediction (best cis-eQTL, best linear unbiased predictor, Bayesian sparse linear mixed model (BSLMM) (Zhou et al., 2013), Elastic-net regression, LASSO regression) were calculated and evaluated for accuracy. The model with largest cross-validation R^2 was chosen for downstream association analyses.

TWAS statistics were calculated using prenatal weights, as well as published adult expression weights (Gusev et al., 2018) calculated from the CMC eQTL dataset for gene and intron level weights (Fromer et al., 2016) and GTEx Cortex expression gene weights (<http://gusevlab.org/projects/fusion/>), LD SNP correlations from the 1000 Genomes European Phase 3 reference panel (as the GWAS used are from European populations) (1000 Genomes Project Consortium et al., 2015), and GWAS summary statistics from CLOZUK +PGC SCZ GWAS (105,318 individuals) (Pardiñas et al., 2018; Ripke et al., 2014) and CHARGE ENIGMA Meta-Analysis ICV GWAS (26,577 individuals) (Adams et al., 2016). TWAS association statistics were Bonferroni corrected per GWAS, gene and intron separately. Additional Fusion parameters include running colocalization by COLOC (-coloc_P 0.05) and increasing the maximum fraction of missing SNPs to be imputed due to ancestry differences between the prenatal eQTLs and the GWAS (-max_impute 0.06).

We created a set of high confident adult brain TWAS gene associations, designated by being significant in 2 of 3 adult brain datasets (CMC, GTEx Cortex, and PsychENCODE). The CMC, GTEx Cortex weights were run with the CLOZUK+ PGC SCZ GWAS and PsychENCODE SCZ TWAS results were downloaded from Gandal et al. (2018b)

Overlap of TWAS hits (± 500 kb) with GWAS significant loci (LD block reported in paper) was assessed for both prenatal TWAS hits and CMC, GTEx cortex, and PsychENCODE adult prefrontal cortex TWAS hits. Novel regions were identified by genes and introns (± 500 k) that did not overlap a GWAS significant loci, and then regions were merged if the gene/intron ± 500 kb overlapped.

Summary-data Bases Mendelian Randomization (SMR)—We performed summary based mendelian randomization (SMR) (Zhu et al., 2016) to identify gene-trait associations by integrating eQTL and GWAS data, as a complementary approach to TWAS. All nominal snp-gene pair prenatal eQTLs and CLOZUK+ PGC SCZ GWAS (Pardiñas et al., 2018) were used as input to SMP. The set based SMR test was run (-smr-multi) using SNPs in the *cis*

window with a $P_{\text{eql}} < 10^{-4}$ ($-\text{peqtl-smr } 1\text{e-}4$) and the associated HEIDI test. For replication of significant TWAS hits, we require genes to have a significant SMR association ($P_{\text{SMR}} < 0.05$) and HEIDI test ($P_{\text{HEIDI}} < 0.05$).

FOCUS fine mapping—TWAS association statistics at genomic risk regions tend to be correlated as a function of linkage and eQTL overlap between predictive models (Mancuso et al., 2019; Wainberg et al., 2017). To prioritize candidate susceptibility genes in our TWAS we performed statistical fine-mapping using FOCUS (Mancuso et al., 2019). FOCUS models the correlation structure induced by LD and overlapping eQTL weights across predictive models and computes posterior probabilities for a gene/intron to explain all observed TWAS association signal at a region. To account for missing gene predictions models, we include gene expression prediction models for genes for genes not predictable from the prenatal brain dataset across 45 tissues measured in 47 reference panels and include the model with the best accuracy among the other tissues. We next computed 90% credible gene-sets by taking genes with largest posterior probability until 90% density was explained.

DATA AND CODE AVAILABILITY

The RNaseq and genotype dataset used for eQTL and sQTL discovery generated in this study are available at dbGaP with accession number: phs001900.

Additional Resources—We have created DevEloPing Cortex Transcriptome (DEPICT) viewer (<https://labs.dgsom.ucla.edu/geschwind/pages/eqtl-browser>), a web resource for viewing summary level eQTL and sQTL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We identify eQTLs and sQTLs specific to human prenatal brain development
- Regulation of expression and splicing differs substantially across development
- Distinct relationships exist between expression, splicing, and disease risk
- Variation in splicing carries as much, if not more, disease risk than expression

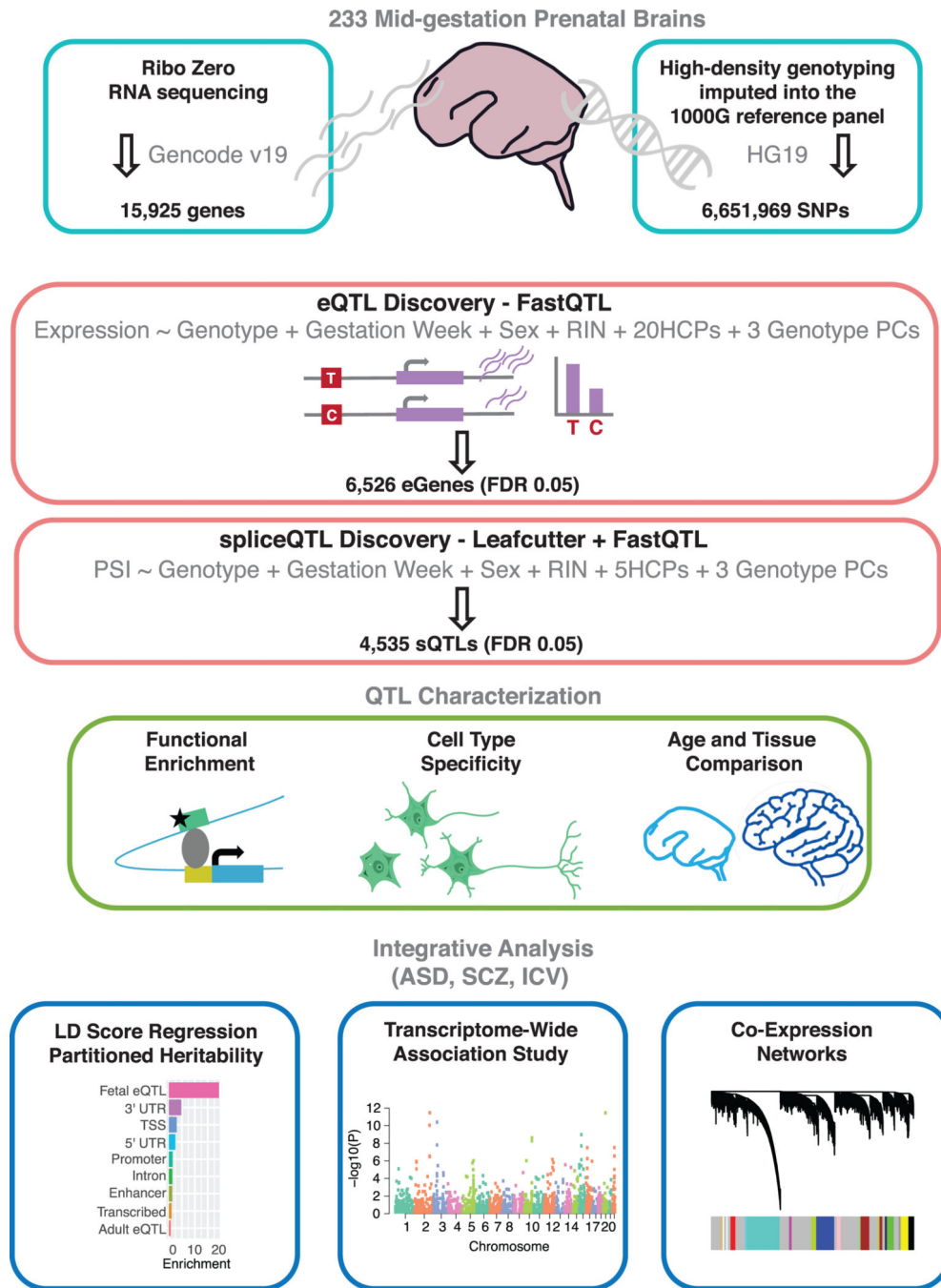


Figure 1. Study Design and Overview of Analysis

RNA-sequencing from 233 prenatal cortical samples was QCed resulting in 201 samples that was integrated with genotypes for eQTL and sQTL discovery. eQTL and sQTL were independently calculated using standard methods of covariate correction (Mostafavi et al., 2013). QTLs were characterized based on functional enrichment, cell type specificity, and compared to mature brain and non-brain tissues. We integrated prenatal brain QTLs with GWAS of neuropsychiatric disorders and other human brain phenotypes, performing

LDSR, TWAS, and WGCNA to identify developmental disease risk and important biological processes under genetic control.
See also Figure S1.

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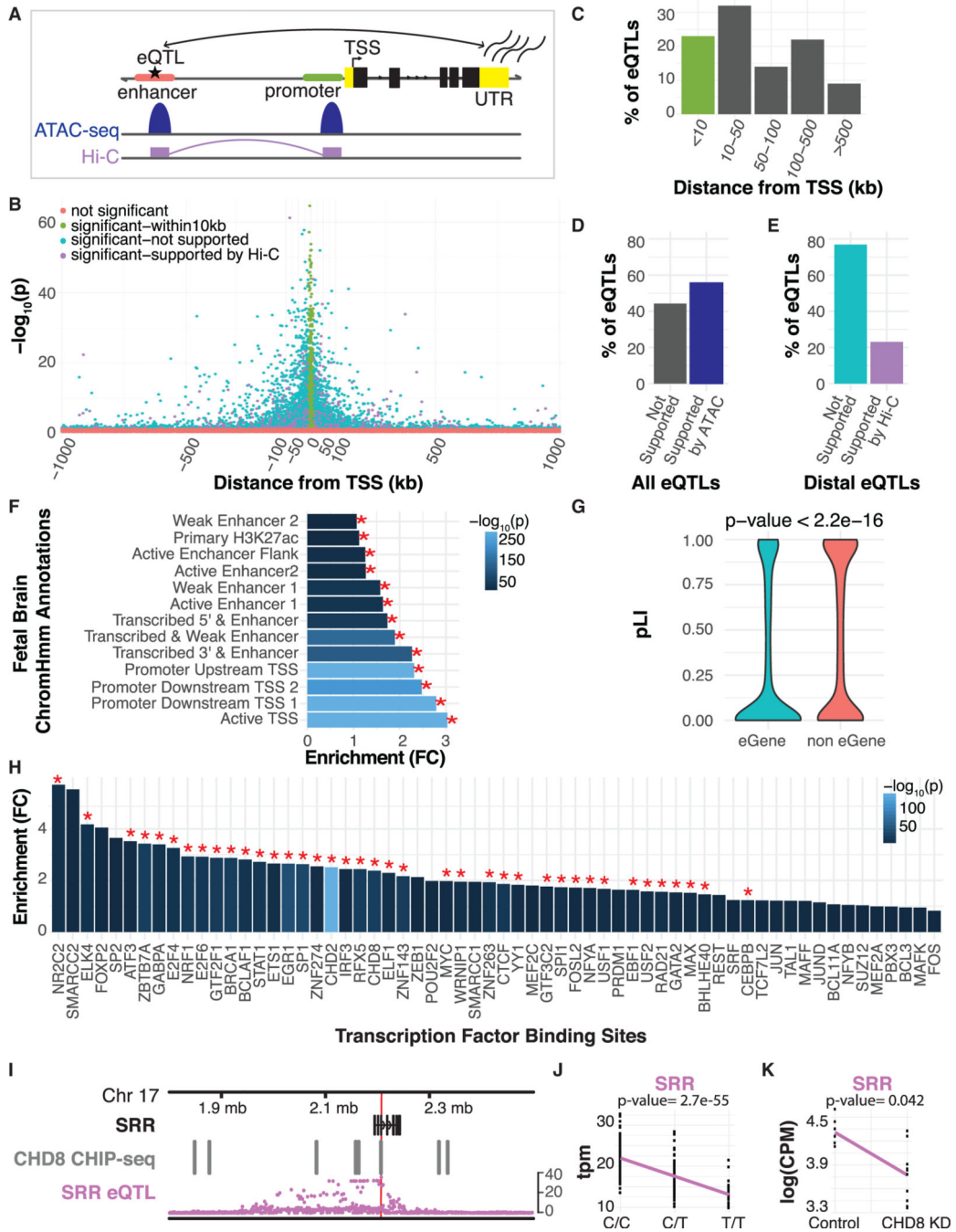


Figure 2. Characterization of eQTLs

(A) A schematic showing that chromatin accessibility (ATAC-seq) and chromatin interactions (Hi-C) are overlapping and support eQTLs in linking genes to distal regulatory elements.

(B) Position of eSNPs in relation to the eGene TSS. Significant eQTLs are annotated based on support from prenatal brain Hi-C and distance from the TSS.

(C) Distribution of all primary and secondary eQTLs. eQTLs that lie within 10 kb of the TSS are colored green, as depicted in (B).

- (D) Fraction of eQTLs with prenatal brain ATAC-seq support.
- (E) Fraction of distal eQTLs (>10 kb from the TSS) with prenatal brain Hi-C support. eQTLs that show Hi-C support are colored purple, while those not supported by Hi-C are colored blue, as depicted in (B).
- (F) Fold enrichment of eSNPs by their distribution within prenatal brain chromatin states, which are listed on the y axis. A red asterisk indicates significance at 5% FDR.
- (G) eGenes are more tolerant to loss of function, as compared with those genes expressed in prenatal brain without significant eQTL (Wilcoxon rank-sum $p < 2.2 \times 10^{-16}$).
- (H) Fold enrichments of eSNPs in TF binding sites (Arbiza et al., 2013). A red asterisk indicates significance at 5% FDR.
- (I) Whole-gene view of *SRR* highlighting the association $-\log^{10}(P)$ for all *cis*-SNPs with expression of *SRR*, the eSNP is denoted by the red line. Additionally, the eSNP falls within a *CHD8* chromatin immunoprecipitation sequencing (CHIP-seq) binding site.
- (J) The eQTL for *SRR* ($p = 2.7 \times 10^{-55}$), showing the distribution of expression values of *SRR* per genotype.
- (K) The expression of *SRR* in control samples versus *CHD8* knockdown (differential expression $p = 0.042$).
- See also Figure S5 and Table S1.

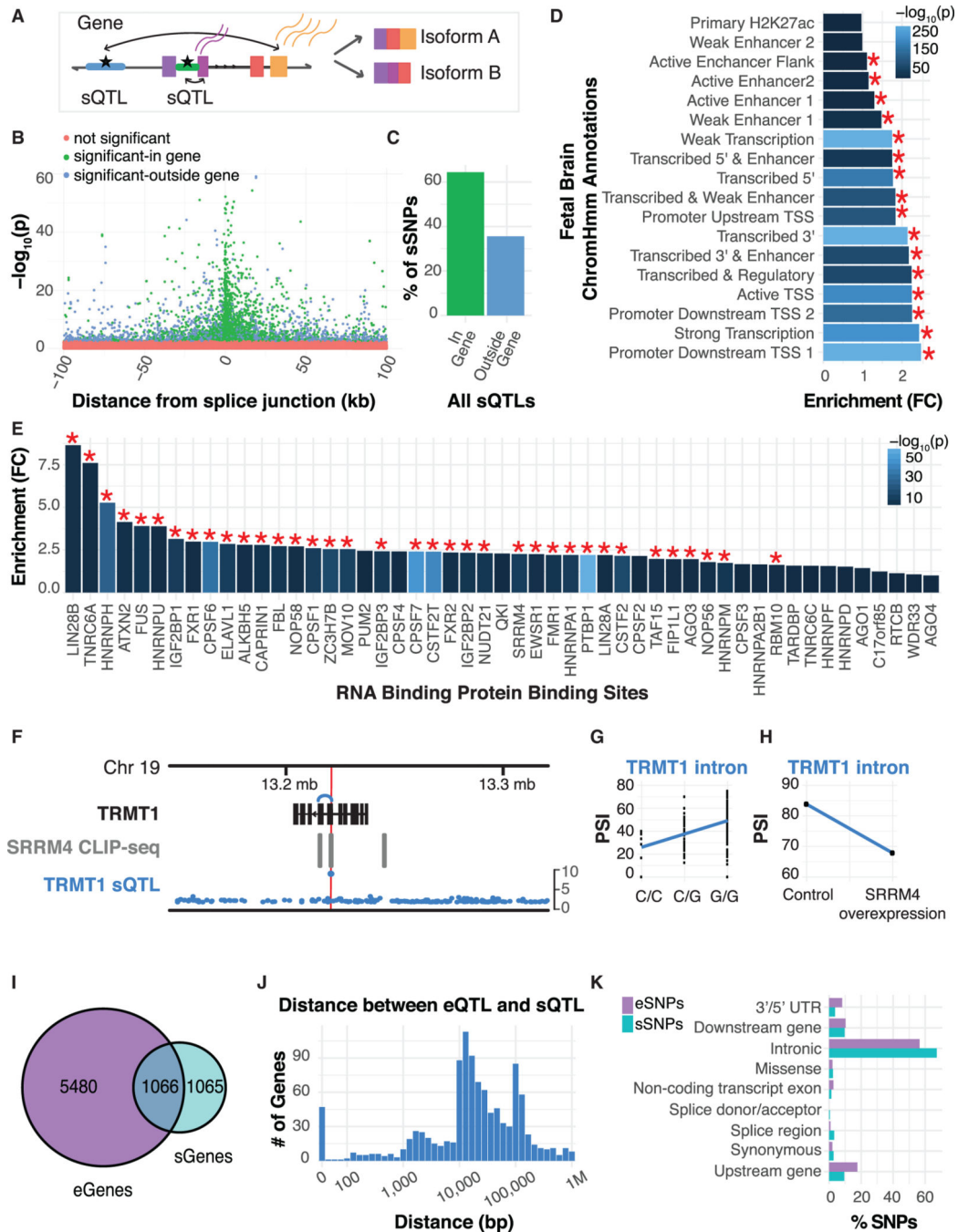


Figure 3. Characterization of sQTLs and Comparison to eQTLs

(A) A schematic of sQTL detection and intron excision when genes are spliced into multiple isoforms.

(B) Position of sQTLs in relation to the splice junction. Significant sQTLs are annotated based on whether the sSNP lies within (green) or outside (blue) of the corresponding sGene.

(C) Fraction of sQTLs where the sSNP lies within versus outside its sGene.

(D) Fold enrichment of sSNPs by their distribution within prenatal brain chromatin states, which are depicted on they axis. A red asterisk indicates significance at 5% FDR.

(E) Fold enrichments of sSNPs in experimentally discovered RBP binding sites (Yang et al., 2015). A red asterisk indicates significance at 5% FDR.

(F) Whole-gene view of *TRMT1* highlighting the association — $\log_{10}(P)$ for all *cis*-SNPs with PSI values for a *TRMT1* intron, the sSNP is denoted by the red line. Additionally, the sSNP falls within a *SRRM4* CLIP-seq binding site.

(G) The sQTL for the *TRMT1* intron, showing the distribution of PSI values of *TRMT1* per genotype.

(H) The average PSI values of the *TRMT1* intron in 3 control samples versus 3 *SRRM4* overexpression samples.

(I) Venn diagram showing the overlap of genes containing an eQTL versus sQTL.

(J) Distribution of the distance in base pairs between eSNP and sSNP for genes harboring both.

(K) Relative proportions of VEP-predicted SNP effects between eSNPs and sSNPs.

See also Figure S5 and Table S2.

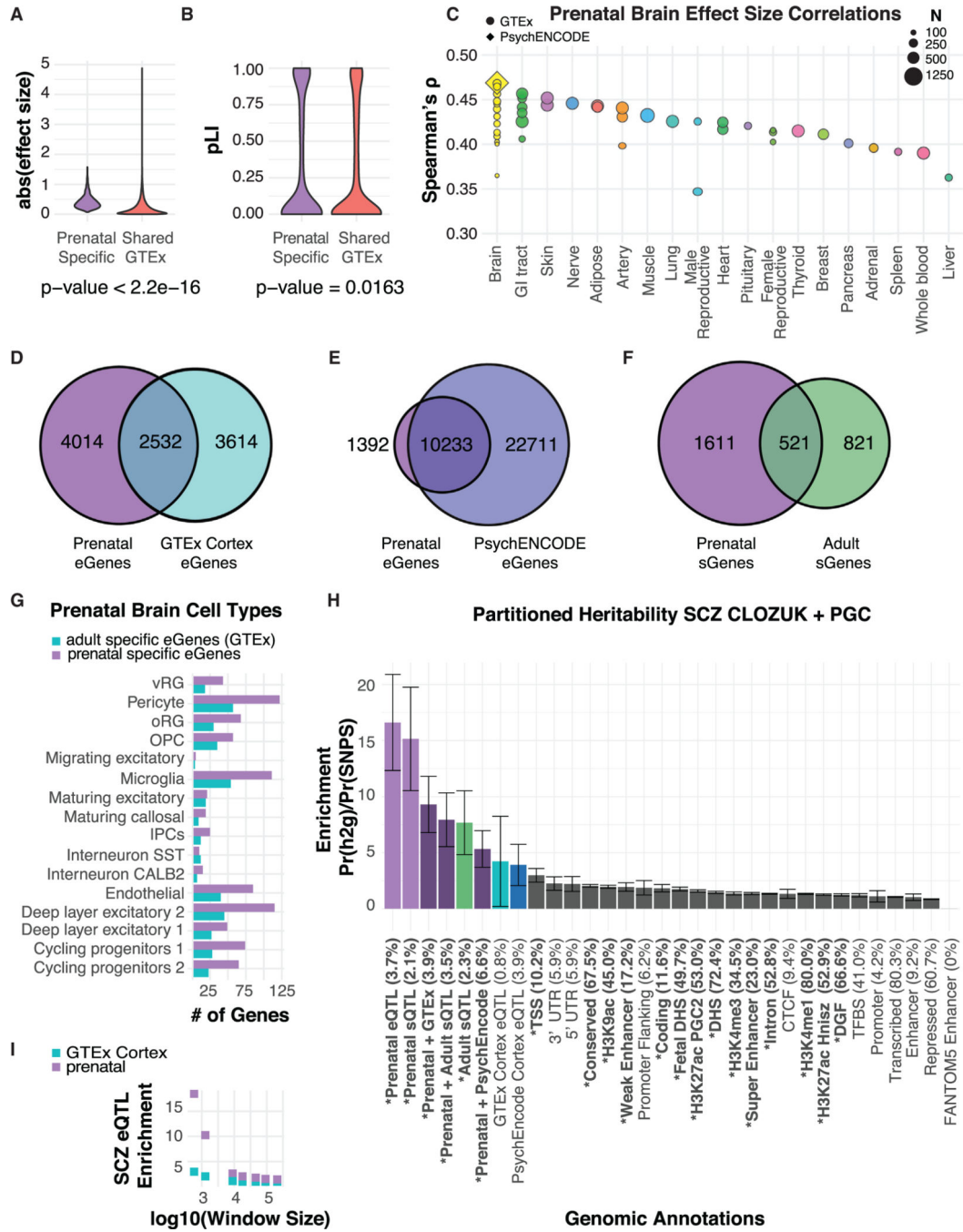


Figure 4. Age Specificity of Brain eQTLs

(A) Distribution of effect size for prenatal-specific eQTLs versus prenatal eQTLs shared in any GTEx tissue (Wilcoxon rank-sum $p < 2.2 \times 10^{-16}$).

(B) Distribution of pLI scores for prenatal-specific eQTLs versus prenatal eQTLs shared in any GTEx tissue (Wilcoxon rank-sum $p = 0.0163$).

(C) Effect size correlations (Spearman's ρ) between significant nominal prenatal eQTLs and corresponding eQTL across tissues grouped by similarity. Tissues from GTEx are denoted

as circles, PsychENCODE prefrontal cortex is denoted by a diamond. The size of each point corresponds to the dataset's sample size.

(D) Venn diagram comparing eGenes discovered in prenatal versus adult cortex from GTEx.

(E) Venn diagram comparing eGenes discovered in prenatal versus adult cortex from PsychENCODE.

(F) Venn diagram comparing sGenes discovered in prenatal versus adult cortex (Takata et al., 2017).

(G) The number of prenatal single cell marker genes containing an eQTL in prenatal-specific eQTLs, GTEx adult brain-specific eQTLs, and those overlapping both datasets. Prenatal, prenatal-specific; adult, adult-specific; both, overlapping and found in both prenatal and adult.

(H) LD score regression enrichments where annotations of prenatal eQTLs and adult eQTLs and sQTLs were added to the baseline annotations. A bold annotation and asterisk indicate significance ($P_{\text{Bonferroni}} < 0.05$) for all annotation categories tested, with the proportion of heritability explained in parenthesis and error bars representing the SE.

(I) LD score regression enrichment of the prenatal cortex and GTEx adult cortex annotations with varying window size around the eSNP.

See also Figure S6.

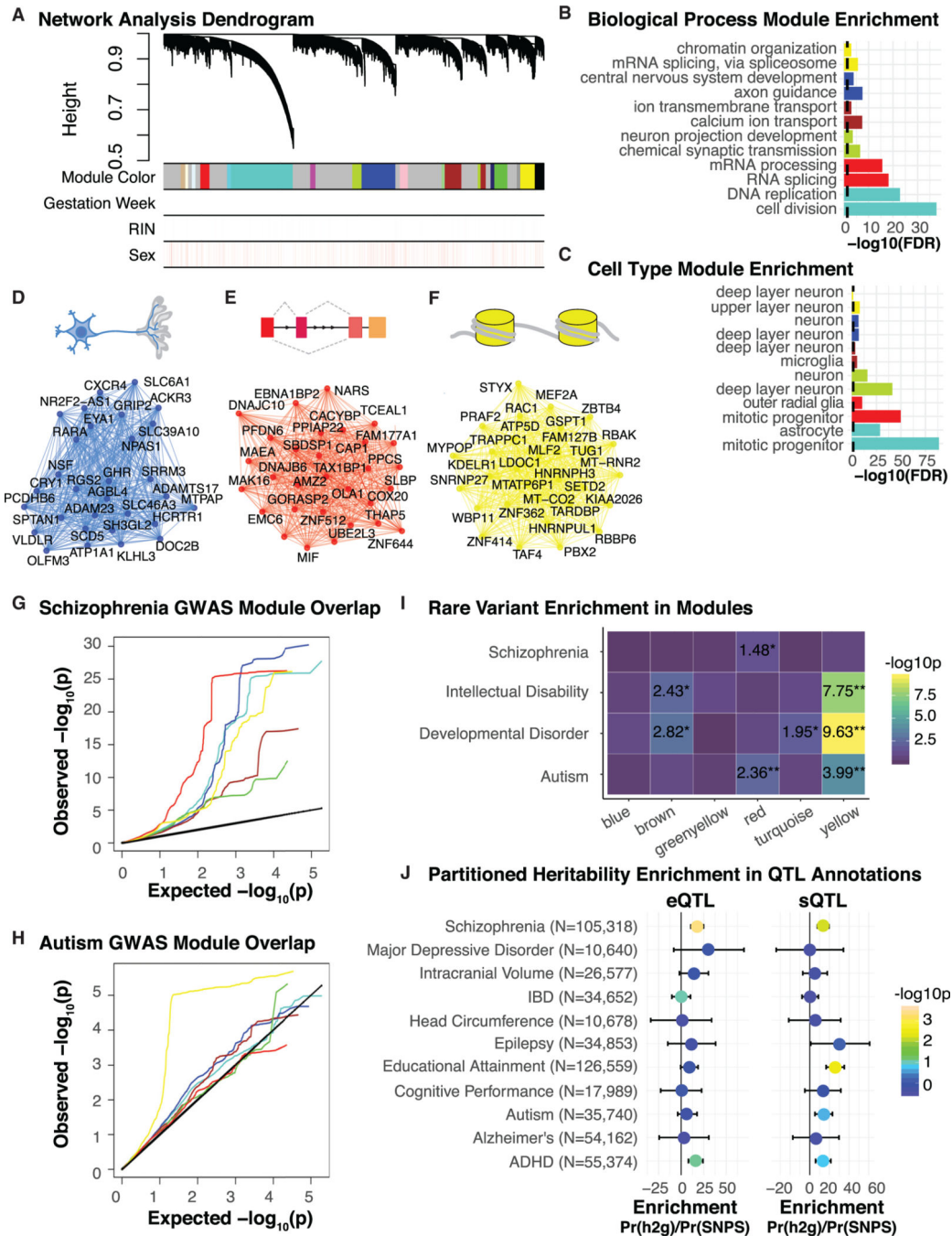


Figure 5. Prenatal Brain Co-expression Networks

(A) Network analysis dendrogram based on hierarchical clustering of genes by their topological overlap, identifies 19 modules. Colored bars below the dendrogram show module membership and expression covariates. Importantly, covariates depicted below the dendrogram are not driving module clustering.

(B) The top 2 Gene Ontology biological process terms enriched for each module. The x axis depicts the $-\log_{10}(\text{FDR})$, the dotted black line indicates significance $-\log_{10}(0.05)$ with all listed categories significant.

- (C) The top 2 cell types enriched for genes within each module. The \times axis depicts the $-\log_{10}(\text{FDR})$, the dotted black line indicates significance $-\log_{10}(0.05)$.
- (D-F) Depiction of module GO term biological process summary and top hub genes along with edges supported by co-expression are shown for the blue (D), red (E), and yellow (F) module. Hub genes are defined by being the top 30 most connected genes based on kME intermodular connectivity.
- (G) Per module QQ plot of SCZ GWAS SNP p values in regulatory regions defined by eQTLs. The red module and the blue module show the most inflation for enrichment of SCZ GWAS hits.
- (H) Per module QQ plot of ASD GWAS SNP p values in regulatory regions defined by eQTLs. The yellow module shows the most inflation for enrichment of ASD GWAS hits.
- (I) Per module rare variant enrichment for SCZ, ASD, ID, developmental disorder. Two asterisks indicate significance after Bonferroni correction, one asterisk indicates nominal significance.
- (J) LD score regression partitioned heritability enrichments for prenatal eQTL and sQTL annotations across multiple GWAS. Enrichments are colored by $-\log_{10}(P)$ and error bars represent SE.
- See also Figure S7 and Table S3.

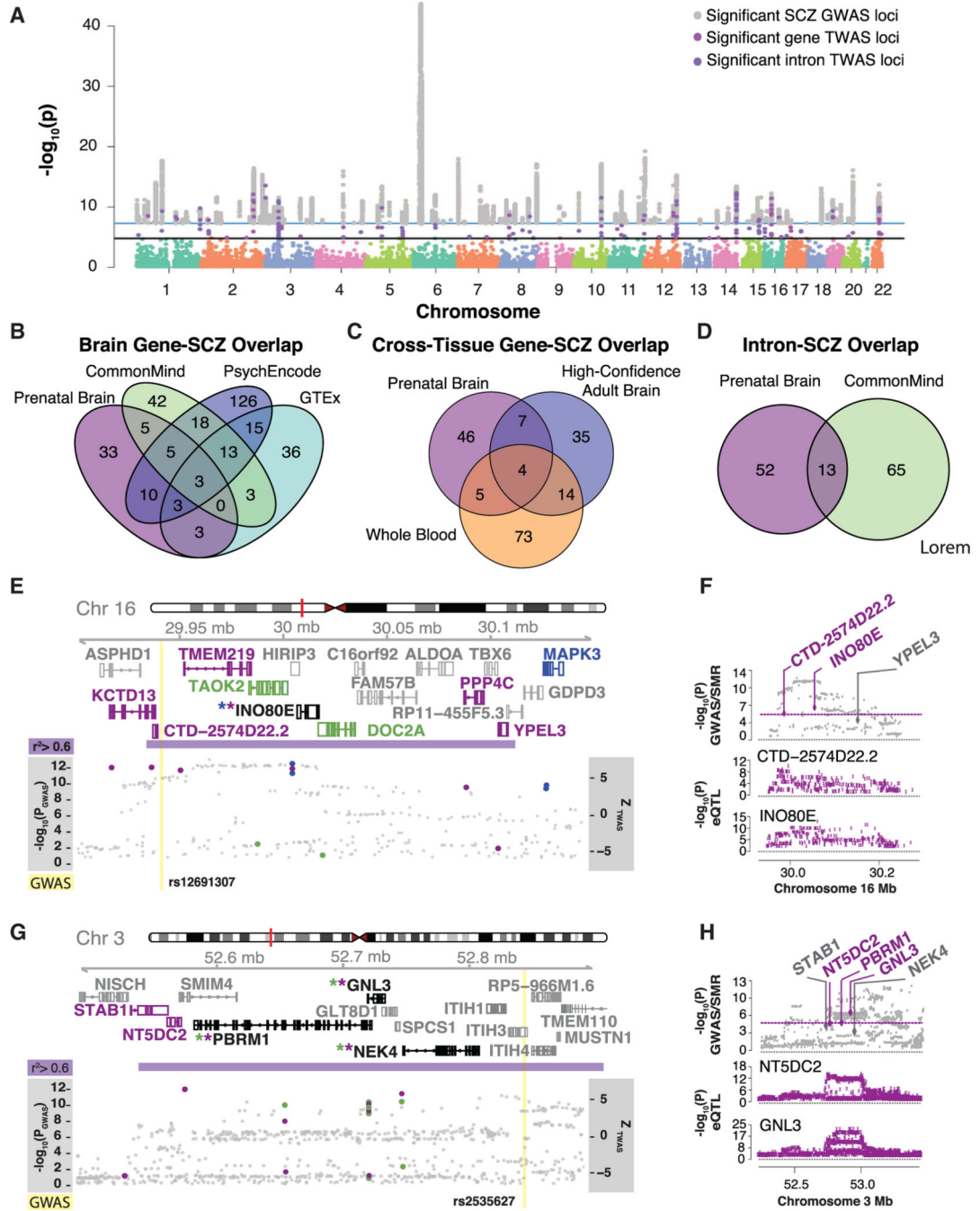


Figure 6. SCZ TWAS

(A) Manhattan plot of TWAS results from genes and intron SCZ associations, highlighting the 62 genes and 91 introns significant at $P_{\text{bonferroni}} < 0.05$, overlaid with the SCZ GWAS significant loci. Key depicts significant GWAS loci (gray), TWAS genes (magenta), and introns (purple).

(B) Overlap of TWAS-significant genes from prenatal brain, adult brain GTEX, adult brain CMC, and adult brain PsychENCODE.

(C) Overlap of TWAS genes from prenatal brain, high-confident adult brain, and whole-blood.

(D) Overlap of TWAS introns from prenatal brain and adult brain.

(E and G) Illustration of two genomic regions on chromosome 16 (E) and on chromosome 3 (G) harboring at least one prenatal TWAS loci. The y axis shows Ensembl gene ID, LD, TWAS results overlapped with GWAS loci $-\log_{10}(P)$; prenatal brain TWAS genes and introns are magenta dots, high-confident adult brain TWAS genes are blue dots, adult brain TWAS introns are green dots, and GWAS SNPs are gray dots) and SCZ GWAS loci (yellow line). Ensembl gene names and gene models are colored by significance in datasets; prenatal brain gene and intron associations (magenta), adult brain intron associations (green), high-confident adult brain gene associations (blue). If a gene is significant in both prenatal and adult it is black with an asterisk colored by the dataset. SCZ GWAS loci are shown in yellow, with the LD block surrounding the SCZ locus in purple defined by $r^2 > 0.6$.

(F and H) Summary data-based Mendelian randomization of prenatal brain genes with SCZ GWAS (F corresponds to the genomic region in E and H corresponds to the genomic region in G). The y axis shows $-\log_{10}(P)$ of SMR and GWAS loci and individual gene eQTL $-\log_{10}(P)$. Genes are colored by passing the 5% FDR threshold for P_{SMR} . See also Tables S4, S5, S6, and S7.

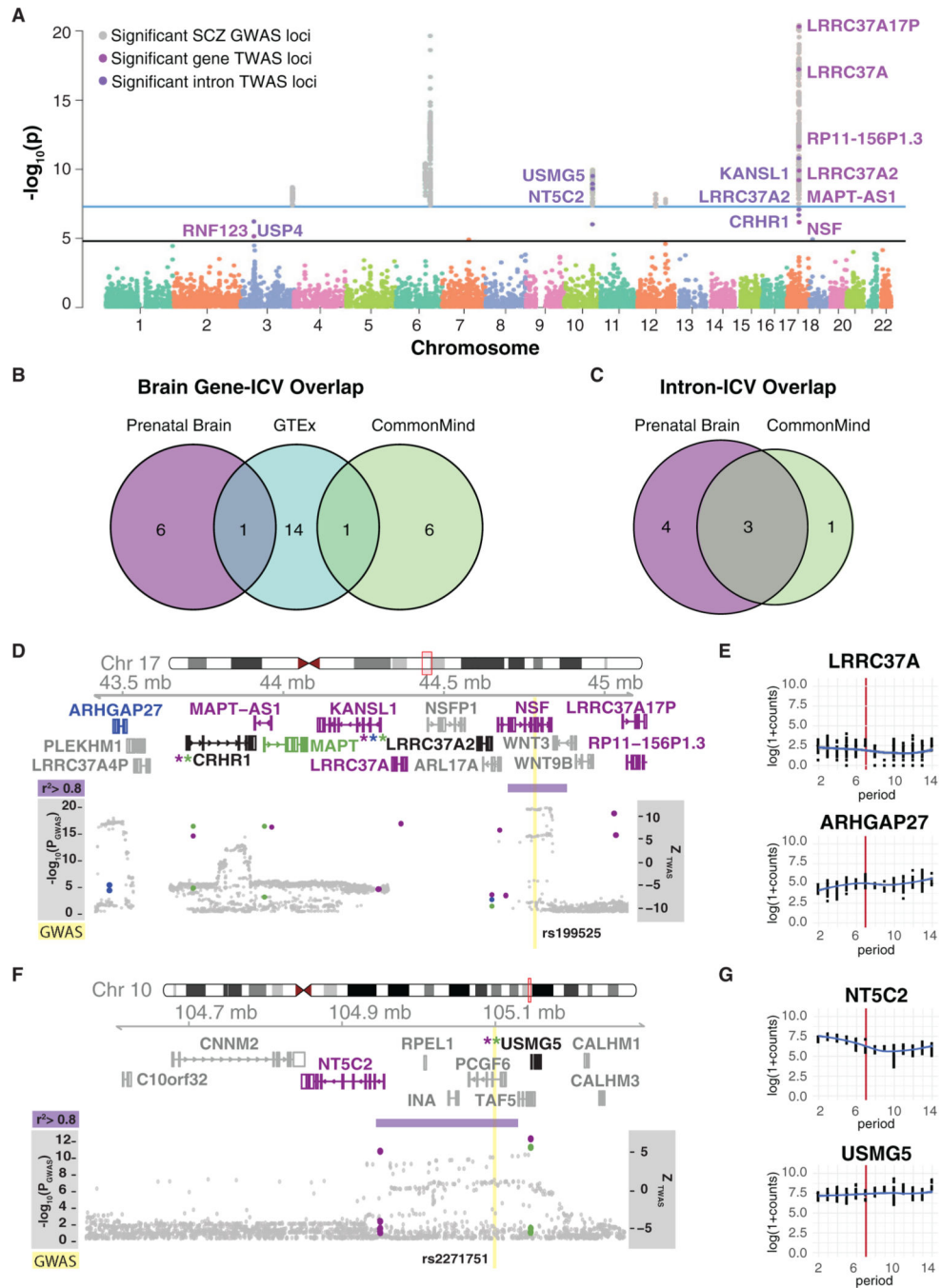


Figure 7. ICV TWAS

(A) Manhattan plot of TWAS results from genes and intron ICV associations, highlighting the 7 genes and 8 introns significant at $P_{\text{bonferroni}} < 0.05$, overlaid with ICV GWAS significant loci. Key depicts significant GWAS loci (gray), prenatal brain TWAS genes (magenta), and introns (purple).

(B) Overlap of TWAS genes from prenatal brain, adult brain from GTEx, and adult brain from CMC.

(C) Overlap of TWAS introns from prenatal brain and adult brain from CMC (Gusev et al., 2018).

(D and F) Illustration of two genomic regions on chromosome 17 (D) and on chromosome 10 (F) harboring at least one prenatal TWAS loci. The y axis shows Ensembl gene ID, LD, TWAS results overlapped with GWAS loci – $\log_{10}(P)$; prenatal brain TWAS genes and introns are magenta dots, high-confident adult brain TWAS genes are blue dots, adult brain TWAS introns are green dots, and GWAS SNPs are gray dots) and ICV GWAS loci (yellow line). Ensembl gene names and gene models are colored by significance in datasets; prenatal brain gene and intron associations(magenta), adult brain intron associations(green), high-confident adult brain gene associations(blue). If the gene is implicated by more than one dataset there is an asterisk colored by the datasets. The ICV GWAS locus is shown in yellow with the LD block in purple defined by $r^2 > 0.8$.

(E and G) Corresponding BrainSpan (BrainSpan, 2013) gene expression trajectories throughout human lifespan of highlighted TWAS genes (E corresponds to significant genes from D and G corresponds to significant genes from F).x axis corresponds to developmental periods defined in Kang et al. (2011), where the red line represents period 8, corresponding to birth.

See also Table S4.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
miRNA easy-mini	QIAGEN	Cat# 217004
DNeasy Blood and Tissue Kit	QIAGEN	Cat# 69506
Stranded TruSeq kit with Ribozero Gold	Illumina	Cat# 20020598
Deposited Data		
Raw and analyzed data: RNA-seq	This paper	dbGaP: phs001900
Raw and analyzed data: DNA	This paper	dbGaP: phs001900
Hg 19 genome build	N/A	ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/technical/reference/phase2_reference_assembly_sequence/
Roadmap Epigenomics 25-state model	Roadmap Epigenomics Consortium et al., 2015	https://personal.broadinstitute.org/jernst/MODEL_IMPUTED12MARKS/
Genecode v 19	Harrow et al., 2012	https://www.genecodegenes.org/human/release_19.html
ENSEMBL Human genome assembly	N/A	ftp://ftp.ensembl.org/pub/release-75/fasta/homo_sapiens/dna/
Developing human cortex Hi-C	Won et al., 2016	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE77565
Developing human cortex ATAC-seq	de la Torre-Ubieta et al., 2018	https://doi.org/10.1016/j.cell.2017.12.014
GTEX v7	GTEX Consortium et al., 2017	https://www.gtexportal.org/home/datasets https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000424.v7.p2
HapMap3	International HapMap Consortium, 2003	ftp://ftp.ncbi.nlm.nih.gov/hapmap/
1000 Genomes Project Phase3	1000 Genomes Project Consortium et al., 2015	ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/
BrainSpan	BrainSpan, 2013	http://brainspan.org/
Transcription factor binding sites	Arbiza et al., 2013	N/A
RNA binding protein binding sites	Yang et al., 2015	https://omictools.com/clipdb-tool
Functional Gene Constraint Scores	Lek et al., 2016	http://exac.broadinstitute.org
Software and Algorithms		
R(v3.2.1)		https://www.r-project.org/
FastQC	N/A	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
RNA STAR v2.4	Dobin et al., 2013	https://github.com/alexdobin/STAR
Picard Tools v1.139	N/A	http://broadinstitute.github.io/picard/
Samtools v1.2	Li et al., 2009	https://samtools.github.io
PLINK 1.9	Chang et al., 2015	https://www.cog-genomics.org/plink2
HTseq Counts	Anders et al., 2015	https://htseq.readthedocs.io/en/release_0.10.0/
VerifyBamID v1.1.2	Jun et al., 2012	https://genome.sph.umich.edu/wiki/VerifyBamID
Plinkseq v0.10	N/A	https://atgu.mgh.harvard.edu/plinkseq/
ComBat	Johnson et al., 2007	https://www.bu.edu/jlab/wp-assets/ComBat/Abstract.html
WGCNA	Langfelder and Horvath, 2008; Zhang and Horvath, 2005	https://cran.r-project.org/web/packages/WGCNA/index.html

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Beagle v4.1	Browning and Browning, 2016	https://faculty.washington.edu/browning/beagle/beagle.html
GATK v3.5	Van der Auwera et al., 2013	https://software.broadinstitute.org/gatk/
HCP	Mostafavi et al., 2013	https://github.com/mvaniterson/Rhcpp
FastQTL	Ongen et al., 2016	http://fastqtl.sourceforge.net
Matrix eQTL	Shabalin, 2012	https://cran.r-project.org/web/packages/MatrixEQTL/index.html
EMMAX	Kang et al., 2008	http://genetics.cs.ucla.edu/emmax_jemdoc/
METAL v3.25.2011	Willer et al., 2010	http://csg.sph.umich.edu/abecasis/Metal/
Leafcutter	Li et al., 2018	http://davidaknowles.github.io/leafcutter/index.html
GREGOR	Schmidt et al., 2015	https://genome.sph.umich.edu/wiki/GREGOR
Variant Effect Predictor	McLaren et al., 2016	https://uswest.ensembl.org/info/docs/tools/vep/index.html
LD Score Regression	Finucane et al., 2015	https://github.com/bulik/ldsc
FUSION	Gusev et al., 2016	http://gusevlab.org/projects/fusion/
FOCUS	Mancuso et al., 2019	https://github.com/bogdanlab/focus/
GenomicRanges Package v1.16.4	Lawrence et al., 2013	http://bioconductor.org/packages/release/bioc/html/GenomicRanges.html
Gviz Package v1.8.4	Hahne and Ivanek, 2016	http://bioconductor.org/packages/release/bioc/html/Gviz.html
biomaRt Package v2.20.0	Durinck et al., 2009	http://bioconductor.org/packages/release/bioc/html/biomaRt.html
BSgenome.Hsapiens.UCSC.hg19 v1.32.0	N/A	http://bioconductor.org/packages/release/data/annotation/html/BSgenome.Hsapiens.UCSC.hg19.html
qvalue Package	Storey and Tibshirani, 2003	https://www.bioconductor.org/packages/devel/bioc/html/qvalue.html
Cqn Package v1.26.0	Hansen et al., 2012	https://bioconductor.org/packages/release/bioc/html/cqn.html
Dplyr Package v0.7.6	N/A	https://cran.rstudio.com/web/packages/dplyr/index.html
Qqman Package v0.1.4	Turner, 2014	https://cran.r-project.org/web/packages/qqman/
AnnotationDbi Package v1.42.1	Pagès et al., 2018	https://www.bioconductor.org/packages/release/bioc/html/AnnotationDbi.html
flashClust Package v1.01–2	Langfelder and Horvath, 2012	https://cran.r-project.org/web/packages/flashClust/index.html
GenomicFeatures Package v1.42.1	Lawrence et al., 2013	https://bioconductor.org/packages/release/bioc/html/GenomicFeatures.html
Igraph Package v1.2.1	Nepusz and Csárdi, 2006	https://cran.r-project.org/web/packages/igraph/index.html
Corrplot v0.84 Package	Wei and Simko, 2017	https://cran.r-project.org/web/packages/corrplot/index.html
SMR	Zhu et al., 2016	http://cnsgenomics.com/software/smr/