Regional heterogeneity in gene expression, regulation and coherence in hippocampus and dorsolateral prefrontal cortex across development and in schizophrenia

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LIEBER INSTITUTE *for* BRAIN DEVELOPMENT
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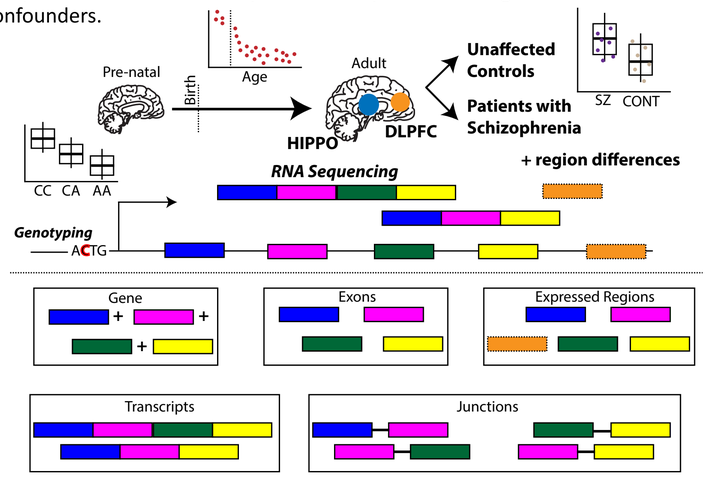
INTRODUCTION

We previously identified widespread genetic, developmental, and schizophrenia-associated changes in polyadenylated RNAs in the dorsolateral prefrontal cortex (DLPFC), but the landscape of hippocampal (HIPPO) expression using RNA sequencing is less well-explored. We performed RNA-seq using RiboZero on 900 tissue samples across 551 individuals (286 with schizophrenia) in DLPFC (N=453) and HIPPO (N=447). We quantified expression of multiple feature summarizations of the Gencode v25 reference transcriptome, including genes, exons and splice junctions. Within and across brain regions, we modeled age-related changes in controls using linear splines, integrated genetic data to perform expression quantitative trait loci (eQTL) analyses, and performed differential expression analyses controlling for observed and latent confounders.



	DLPFC	HIPPO	total
adult (age >= 18)	374	370	744
prenatal	29	28	57
0 <= age < 18	50	49	99
total	453	447	900

Schizophrenia disorder cases

	DLPFC	HIPPO	total
adult	152	132	284
prenatal	0	0	0
0 <= age < 18	1	1	2
total	153	133	286

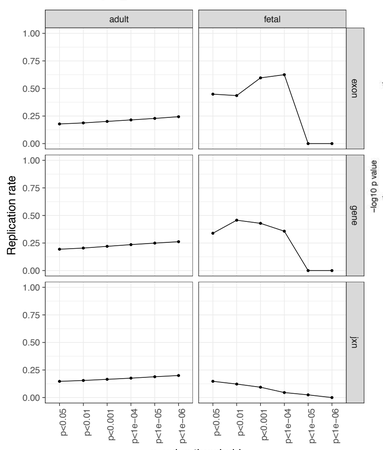
Non-psychiatric controls

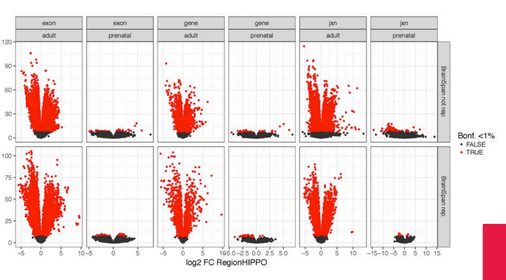
	DLPFC	HIPPO	total
adult	222	238	460
prenatal	29	28	57
0 <= age < 18	49	48	97
total	300	314	614

METHODS

Focus on being conservative: established processing methods, strict cutoffs, using replication when possible, adjust for RNA quality degradation confounding, avoid potential batch effects and take into account correlation at the individual level.

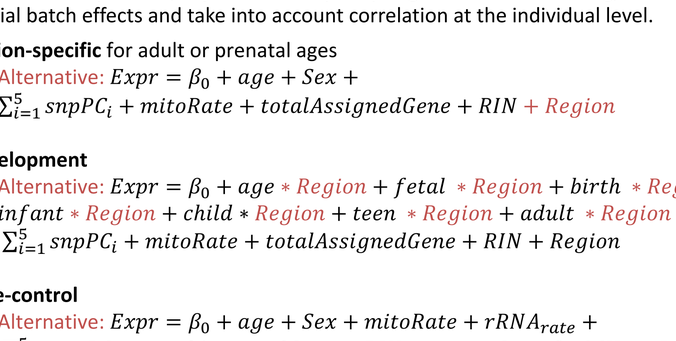
- **Region-specific** for adult or prenatal ages
  - Alternative: $Expr = \beta_0 + age + Sex + \sum_{i=1}^{5} snpPC_i + mitoRate + totalAssignedGene + RIN + Region$
- **Development**
  - Alternative: $Expr = \beta_0 + age * Region + fetal * Region + birth * Region + infant * Region + child * Region + teen * Region + adult * Region + Sex + \sum_{i=1}^{5} snpPC_i + mitoRate + totalAssignedGene + RIN + Region$
- **Case-control**
  - Alternative: $Expr = \beta_0 + age + Sex + mitoRate + rRNA_{rate} + \sum_{i=1}^{5} snpPC_i + totalAssignedGene + RIN + regionSpecificQSVs + Diagnosis$



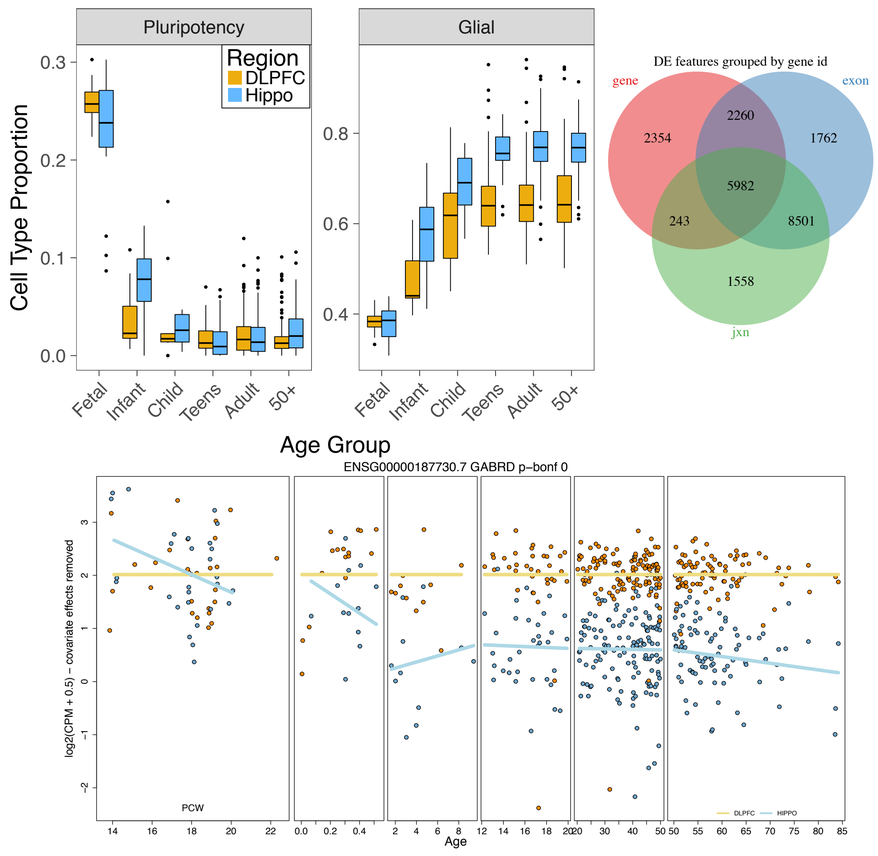


Replication using BrainSpan www.brainspan.org

REGION-SPECIFIC (PRENATAL or ADULT)



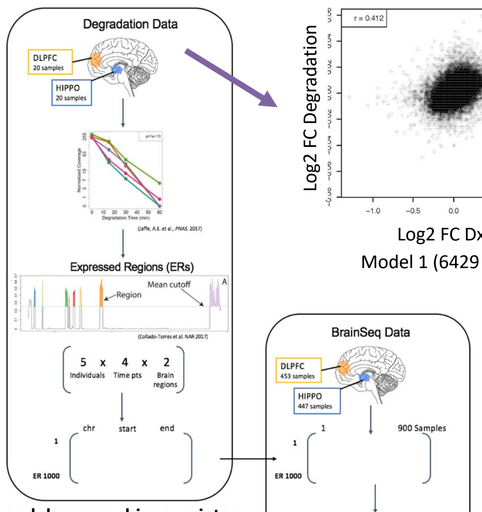
DEVELOPMENT



RNA DEGRADATION ADJUSTMENT: qSVA

SCZD diagnosis is confounded by RNA degradation. To remove this confounder we use the *quality* Surrogate Variable Analysis framework that requires two matched datasets:

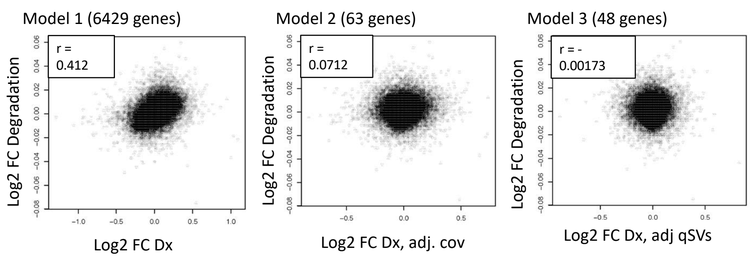
- degradation data
- case-control data



Model 1. Naïve model
$E[Y] = \beta_0 + \beta_1 Dx$

Model 2. Added RNA-quality and demographic covariates
$E[Y] = \beta_0 + \beta_1 Dx + \beta_2 age + \beta_3 sex + \beta_4 mitoRate + \beta_5 rRNArate + \beta_6 geneRate + \beta_7 RIN + \sum_{i=1}^{5} \gamma_i snpPC_i$

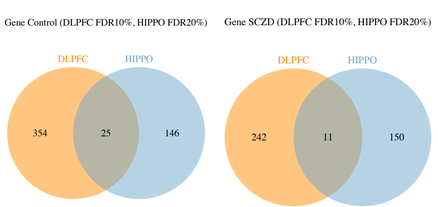
Model 3. Added qSVs
$E[Y] = \beta_0 + \beta_1 Dx + \beta_2 age + \beta_3 sex + \beta_4 mitoRate + \beta_5 rRNArate + \beta_6 geneRate + \beta_7 RIN + \sum_{i=1}^{5} \gamma_i snpPC_i + \sum_{i=1}^{k} Z_i qSV_i$

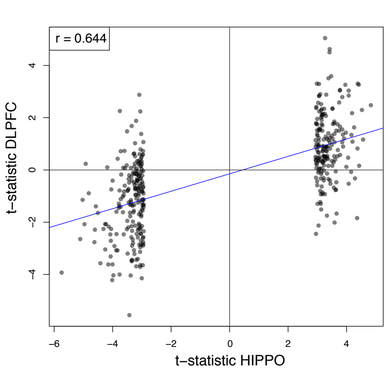


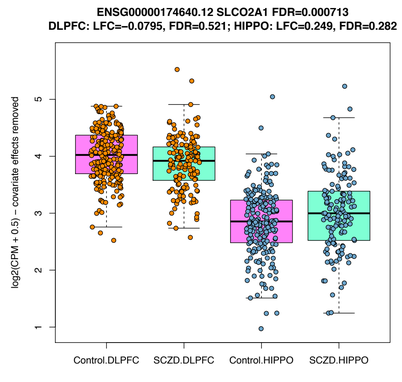
SCHIZOPHRENIA VS NON-PSYCHIATRIC CONTROLS

Results suggest regional heterogeneity of the molecular correlates of schizophrenia diagnosis:

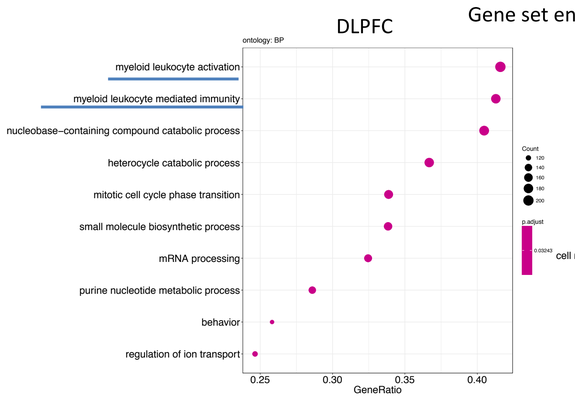
- 48 DE genes in hippocampus, 245 in DLPFC (FDR <5%)
- Reduced overlap among brain regions
- 111 brain-region dependent SCZD DEGs

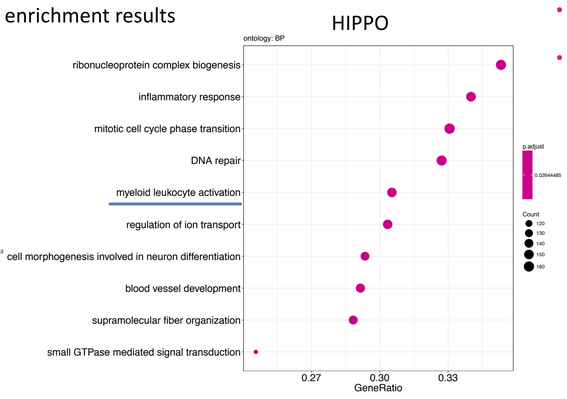




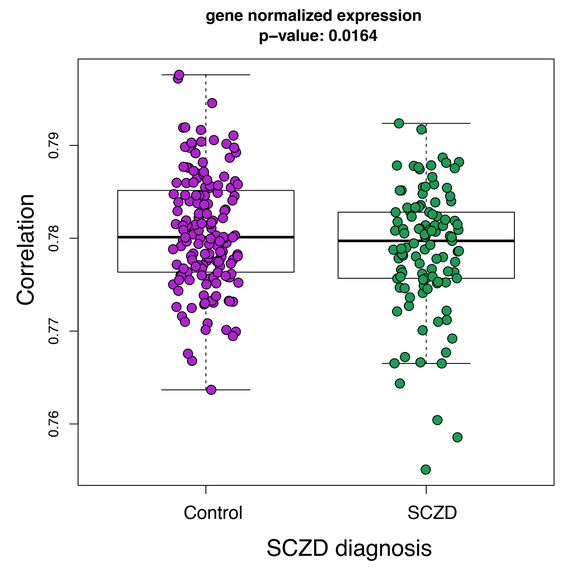


Gene set enrichment results





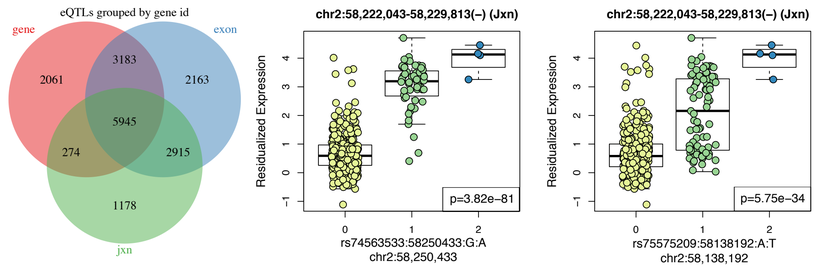
COHERENCE



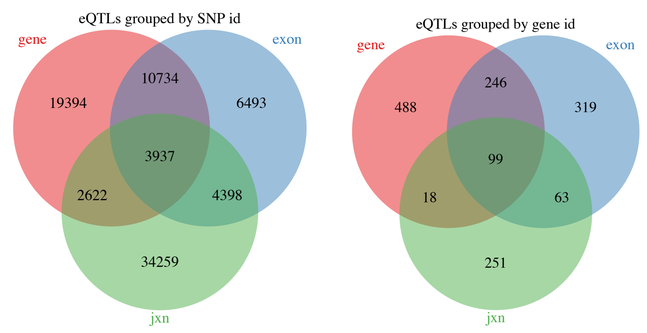
We found significant decreased correlation (p<0.05) at all expressed featured levels in SCZD vs controls (n=265).

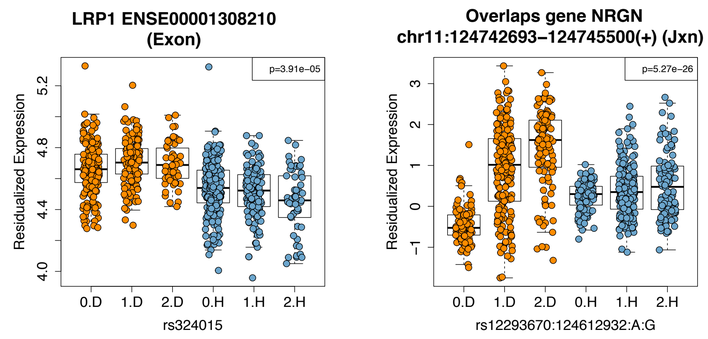
eQTL associations

HIPPO eQTLs: 11,237,357 eQTL associations (FDR <1%) across genes, exons and junctions corresponding to 17,719 genes. Includes 60 GWAS PCG2 SCZD risk loci.



Region dependent eQTLs: 205,618 region-dependent eQTL associations (FDR <1%) corresponding to 1,484 genes. Includes 3 GWAS PCG2 schizophrenia risk loci.





KEY FINDINGS

- Extensive diverging gene expression levels between the DLPFC and HIPPO across neurotypical brain development, beginning from similar expression in prenatal life to widespread differences postnatally and in adulthood.
- Largely disjoint genes differentially expressed in schizophrenia between the DLPFC and HIPPO that point to unique molecular processes and pathways.
- Decreased coherence between the DLPFC and HIPPO in schizophrenia observed across multiple gene expression feature summarizations.
- The largest and most extensive HIPPO expression quantitative trait loci (eQTL) database to date, and presenting these results in our user-friendly web resource: http://eqtl.brainseq.org/phase2/eqtl.
- Widespread eQTLs to risk variants to both HIPPO and DLPFC from the latest and largest genome-wide association study (GWAS) for schizophrenia. We found eQTL evidence for 124/163 loci (76.6%), providing new clues to the molecular consequences of schizophrenia genetic risk. This represents more than a 50% increase in eQTLs among GWAS significant SNPs, further highlighting the incompleteness of prior analyses based only on DLPFC.
- Potential need to have regionally targeted therapies for schizophrenia.

REFERENCES & ACKNOWLEDGEMENTS

- Jaffe et al., qSVA framework for RNA quality correction in differential expression analysis, PNAS, 2017
- Jaffe et al., Developmental And Genetic Regulation Of The Human Cortex Transcriptome In Schizophrenia, Nature Neuroscience, 2018
- Pre-print available at https://www.biorxiv.org/content/early/2018/09/26/426213

Funding:

- NIH R21-MH109956-01 awarded to A.E.J.
- BrainSeq Consortium
- LIBD @lieberinstitute

We are hiring in the Andrew E. Jaffe LIBD Data Science team!

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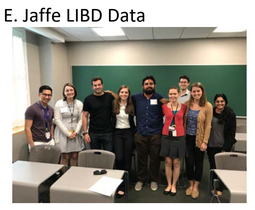

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