Transcriptome and epigenome landscape of human cortical development modeled in brain organoids

Anahita Amiri[1*], Gianfilippo Coppola[1*], Soraya Scuderi[1*], Feinan Wu[1*], Tanmoy Roychowdhury[2*], Fuchen Liu[3], Sirisha Pochareddy[3], Yurae Shin[3,4], Alexias Safi[5], Lingyun Song[5], Ying Zhu[3], André M. M. Sousa[3], The PsychENCODE Consortium, Mark Gerstein[6], Gregory E. Crawford[5], Nenad Sestan[3,7], Alexej Abyzov[2,+], Flora M. Vaccarino[1,3,7,+]

*Equal contribution author
[1]Child Study Center, Yale University, New Haven, CT 06520
[2]Department of Health Sciences Research, Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905
[3]Department of Neuroscience, Yale University, New Haven, CT 06520
[4]National Research Foundation of Korea, South Korea
[5]Department of Pediatrics, Division of Medical Genetics, Duke University, Durham, NC 27708
[6]Department of Molecular Biology and Biophysics, Yale University, New Haven, CT 06520
[7]Kavli Institute for Neuroscience, Yale University, New Haven, CT 06520

[+]Correspondence to Dr. Vaccarino and Dr. Abyzov

INTRODUCTION: The human cerebral cortex has undergone an extraordinary increase in size and complexity during mammalian evolution. Cortical cell lineages are specified in the embryo, and genetic and epidemiological evidence implicate early cortical development in the etiology of neuropsychiatric disorders such as autism spectrum disorder (ASD), intellectual disabilities, and schizophrenia. Most the disease-implicated genomic variants are located outside genes, and the interpretation of non-coding mutations is lagging behind due to limited annotation of functional elements in the non-coding genome.

RATIONALE: We set out to discover gene regulatory elements and chart their dynamic activity during prenatal human cortical development, focusing on enhancers, which carry most of the weight upon regulation of gene expression. We longitudinally modelled human brain development using human induced pluripotent stem cells (hiPSC)-derived cortical organoids and compared organoids to isogenic fetal brain tissue.

RESULTS: Fetal fibroblast derived hiPSC lines were used to generate cortically patterned organoids, and to compare epigenome and transcriptome to isogenic fetal brains and external datasets. Organoids model cortical development between 5 and 16 post conception weeks, thus enabling us to study transitions from cortical stem cells to progenitors to early neurons. The greatest changes occur at the transition from stem cells to progenitors. The regulatory landscape encompasses a total set of 96,375 enhancers linked to target genes, with 49,640 enhancers being active in organoids but not in mid-fetal brain, suggesting major roles in cortical neuron specification. Enhancers that gained activity in the human lineage are active in the earliest stages of organoid development, when they target genes that regulate the growth of radial glial cells.

Parallel WGCNA analysis of transcriptome and enhancer activities defined a number of modules of co-expressed genes and co-active enhancers, following just six and four global temporal patterns which we refer to as supermodules, likely reflecting fundamental programs in embryonic/fetal brain. Correlations between gene expression and enhancer activity allowed stratifying enhancers into two categories: activating regulators (A-regs) and repressive regulators (R-regs). Several enhancer modules converged with gene modules, suggesting that co-expressed genes are regulated by enhancers with correlated patterns of activity. Furthermore, enhancers active in organoids and fetal brains were enriched for ASD *de novo* variants disrupting binding sites of homeodomain, Hes1, NR4A2, Sox3, and NFIX transcription factors.

CONCLUSION: We validated hiPSC-derived cortical organoids as a suitable model system to study gene regulation in human embryonic brain development, evolution and disease. Our results suggest that organoids may reveal how noncoding mutations contribute to ASD etiology.

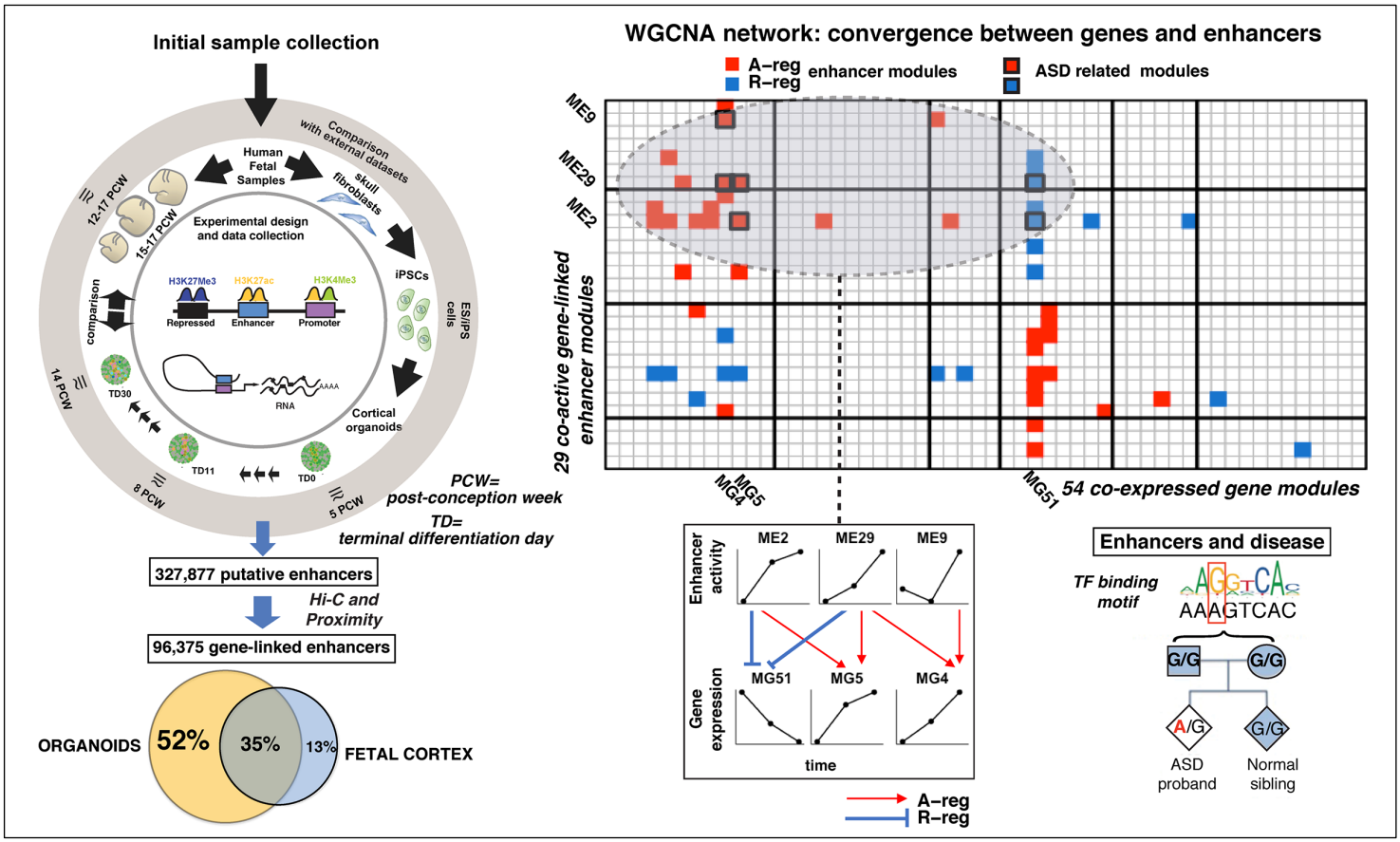

FIGURE CAPTION. **Summary of the study, analyses, and main results.** Data were generated for iPSCs-derived human telencephalic organoids and isogenic fetal cortex. Organoids modelled embryonic/early fetal cortex and show larger enhancer's repertoire. Enhancers could be divided into activators and repressors of gene expression. We derived networks of modules and supermodules with correlated gene and enhancer activities, some of which were implicated in autism spectrum disorders (ASD).