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Transcription Factor-Driven Production of Midbrain Dopaminergic Neurons from Human iPS Cells and Psychiatric Disease Risk Modeling

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Abnormalities in dopaminergic neurotransmission are important pathophysiological hallmarks of several psychiatric and neurological disorders. Derivation of neurons from human iPS cells (hiPSC) enables modeling of typical neurodevelopment and disease processes in an isogenic manner, but techniques for producing hiPSC-derived dopaminergic neurons suffer from variable reproducibility and yield heterogenous cell populations. We sought to develop a protocol for generating pure and homogenous midbrain dopaminergic neurons from hiPSCs through transduction of the lineage-promoting transcription factors *ASCL1, LMX1b,* and *NURR1* via a doxycycline-inducible lentivirus vector, combined with antibiotic selection. Induced dopaminergic neurons (iDANs) show robust increases in the expression of many marker genes of midbrain dopaminergic identity. Across five donors and multiple replicate experiments, about 90% of all cells produced are positive for *tyrosine hydroxylase*, the rate-limiting enzyme of dopamine biosynthesis. Electrophysiologic profiling shows that iDANs demonstrate several functional hallmarks of *in vivo* dopaminergic neurons. By transcriptomic characterization of iDANs, isogenic hiPSCs, and post-mortem tissues, we find further evidence that iDANs possess an early developmental, midbrain dopaminergic neuron identity. Finally, generation of isogenic glutamatergic and GABAergic neurons as well enabled analysis of differential enrichment of gene expression profiles across three major neurotransmitter systems for distinct psychiatric disorder risk genes. In conclusion, these results indicate that hiPSC-derived dopaminergic neurons resemble their *in vivo* counterparts in several important ways and may serve as a valuable model for studying the effects of psychiatric and neurological risk loci on neuronal subtype-specific gene expression and phenotypes.

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