Scaling Human Neurons for HTS to identify Molecules that rescue the Wildtype Neuronal Phenotype that is disrupted in Autism Spectrum Disorder

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental syndrome that is characterized by deficits in social and communication skills with restricted and repetitive behavior. ASD has a high prevalence rate of 1 in 59 children and has no effective drug to treat the core symptoms. De novo germline mutations often play an important role in ASD and several risk loci and genes have been identified in the last decade. These mutations ranged from copy number variants to small insertion/deletion and single nucleotide variants. Genotyping and exome sequencing of the Simons Simplex Collection identified 65 ASD risk genes that encode either chromatin regulators or synaptic proteins (Sanders et al., 2015). The complex etiology of ASD and unavailability of human neurons remain a major hurdle in understanding the pathophysiology and for testing new drug candidates.

Generation of neurons from patient derived induced Pluripotent Stem Cells (iPSCs) can overcome these challenges. In this study, we used three different genetic variants of iPSCs derived from CRISPR editing to emulate the mutations found in ASD patient cells. Pluripotency of these cells was confirmed by immunostaining and neurons were generated using previously established protocol (Sridharan et al., 2019). Induced neurons were grown in 384 well plates, neurite outgrowth staining performed, and high content images acquired. Significant phenotypic differences were observed between the genetic variants of ASD compared to parent in terms of neuron number, number of neurites and neurite length per neuron. This assay is now being miniaturized to 1356 well plates compatible with high throughput screening. Thus, neuronal phenotypic changes induced by different mutations in ASD may be assessed against large libraries of small molecules and or biologics which ultimately may lead to pharmacological interventions.

Rationale

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**ASD Risk Genes**

- Protein-Protein Interaction Networks in ASD: 65 ASD genes identified with TADA FDR 0.1 were submitted as seeds to form a DAPPLE PPI network. Protein-protein interactions are shown as gray lines (edges) and additional genes are pulled into the network to form indirect connections. These 65 genes form a network of protein-protein interactions composed of two sub-networks that are enriched for genes that encode either chromatin regulators or synaptic proteins.

- (Adapted from Sanders et al., 2015)

**Neural Induction**

- a) Timeline of Ngn2-induction strategy for differentiating hPSCs into neurons.
- b) Representative phase contrast photomicrograph of Human induced Pluripotent Stem Cells- ADNP p.Arg832Lys*, FOXP1 p.Arg252*, C1577, Parental line- ASFS003, SHANK3-C1455-Clone 10 and SHANK3-C1455-Het at different days of neural induction (all magnification 4x).

**Assay Development**

- **High Content Imaging**
- Representative CellInsight fluorescent photomicrographs of hiPSCs stained with Hoechst, OCT4 in the nucleus and their composite which confirms pluripotency.

**Compound Testing**

- a) Dose Response Curve of iNs from a. parent hiPSC and b. CRISPRRed genetic variant (ADNP) treated with different compounds. Compounds tested at a concentration of 10 μM (N of 1 experiment, n=2 wells / concentration tested).

**Conclusion**

Human iPSCs parent and CRISPRRed genetic variants of ASD were delivered to MSSM, scaled up and induced to glutamatergic neurons, validating the established neural induction protocol for different hiPSCs. iNs scaled to an assay ready format compatible for HTS demonstrated significant phenotypic differences between parent and the genetic variants of ASD in terms of neurite outgrowth. Upon testing with different compounds, iNs were responsive correlating to the concentrations of compounds being tested. This assay is being miniaturized to test against libraries with compounds that can affect the mutant phenotype. Furthermore, this assay can be used to investigate gene correction strategies restoring the wildtype neuronal phenotype.