Generation and Characterization of Sex-Specific H-IPSC Derived Neural Progenitor Cells for Alzheimer's Disease Studies

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Research Question: Do Neural Progenitor Cells differentiated from the same hiPSC cell lines of two sexes develop and retain key characteristics of each cell type?

Introduction and Background:

Neural Progenitor Cells (NPCs) are cells that maintain pluripotency and have the capacity to differentiate into cells of the Central Nervous System. They are commonly converted from human induced Pluripotent Stem Cells (hiPSCs).

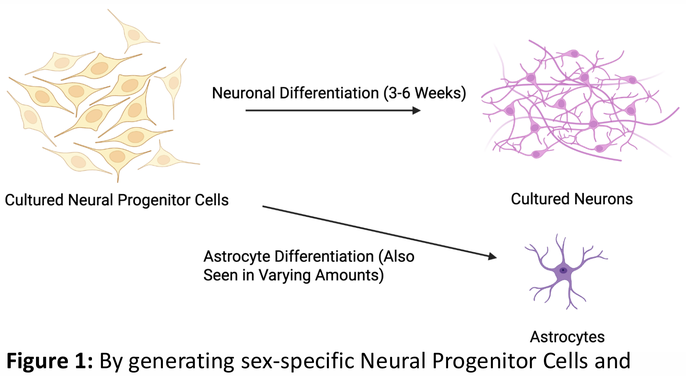

Figure 1: By generating sex-specific Neural Progenitor Cells and characterizing these NPCs using immunofluorescence, we would be able to develop neurons and glial cells that will enable the study of the sex-specific disparities in the onset and progression of Alzheimer's disease.

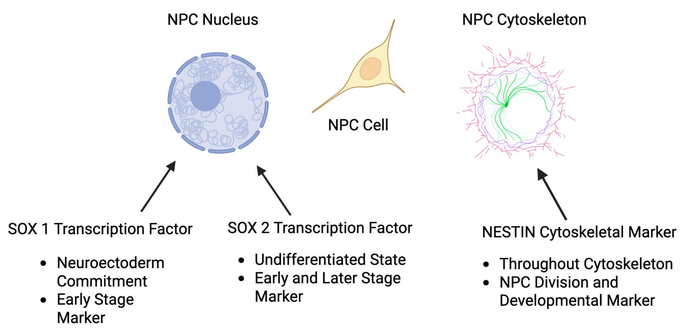

Figure 2: NPCs used in this experiment should express NPC-specific markers, SOX1, SOX2 and Nestin that allow them to be further differentiated into Neurons.

Results:

Male (XY)

Staining for the Male (XY) Cell Line for all 3 Markers

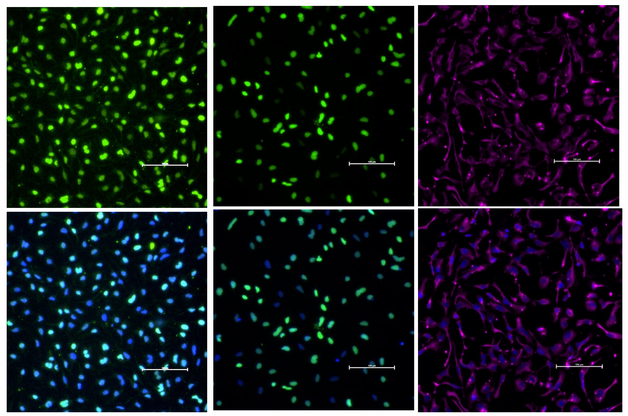

Figure 3: These images show the staining for the male line for markers specified previously. These cells are shown with and without the DAPI nucleus stain (Mag: 20x). The specifics of each NPC marker is specified below.

Female (XX)

Staining for the Female (XX) Cell Line for all 3 Markers

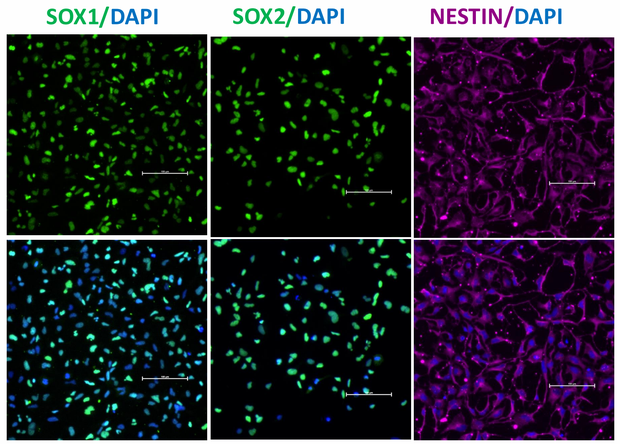

Figure 4: The following images show the staining for the female line. SOX1 demonstrates the prevention of premature differentiation, and SOX2 demonstrates identity as a neural stem cell along with the prevention of differentiation. NESTIN demonstrates NPC divisional and developmental characteristics within the cytoskeleton.

Staining of Neurons Derived from the Same Sex-Specific Lines as the NPCs

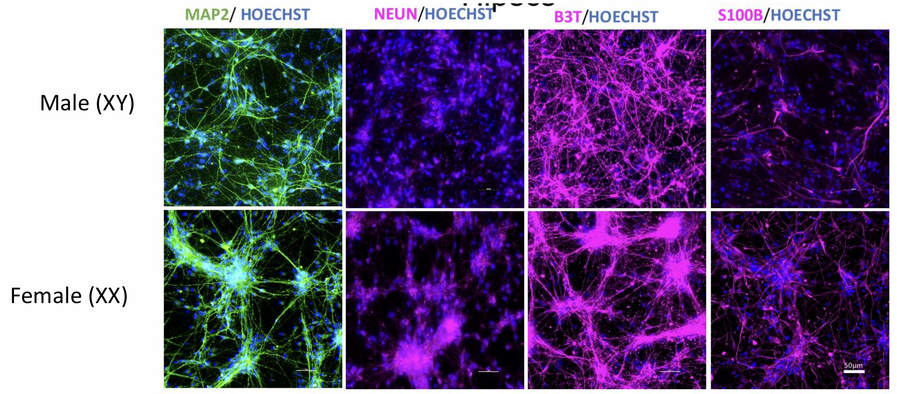

Figure 5: The above images of neurons were characterized for TUJ1/B3T, a pan-neuronal marker, Map2, a dendritic marker, NEUN, a protein marker demonstrating neuronal maturity, and S100B, an astrocyte marker. These neurons were differentiated using the same NPC lines as above. (Mag: 20X)

Conclusions:

NPC Cell Characterization

The expression of all 3 markers was observed in both cell lines, ultimately indicating normal development and normal characteristics of NPCs. Altogether, we can conclude that both sexes retain NPC characteristics that SOX1, SOX2, and NESTIN help visualize.

Neuronal Characterization:

Overall, the neuronal staining demonstrated sustained expression for all markers between the male and the female lines. Astrocyte presence was seen in both lines, and neuronal maturity was also demonstrated, reaffirming successful differentiation.

Future Research: Many other sex-dependent factors can play a role in the development of Alzheimer's. Microglia are the glial cells that initiate an inflammatory response to amyloid-beta plaques, and astrocytes also play an important role with an inflammatory loop they have with microglia. hiPSCs can be differentiated to further study this with the addition of synthetic amyloid-beta plaques.

References:

Knittel, J., Srinivasan, G., Frisch, C., Brookhouser, N., Raman, S., Essuman, A., & Brafman, D. A. (2022). A microcarrier-based protocol for scalable generation and purification of human induced pluripotent stem cell-derived neurons and astrocytes. *STAR Protocols*, *3*(3), 101632. https://doi.org/10.1016/j.xpro.2022.101632

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Images created with Biorender.com

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