Characterization of striatal neurons derived from >140 CAG iPSCs for Huntington's Disease modeling

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Abstract

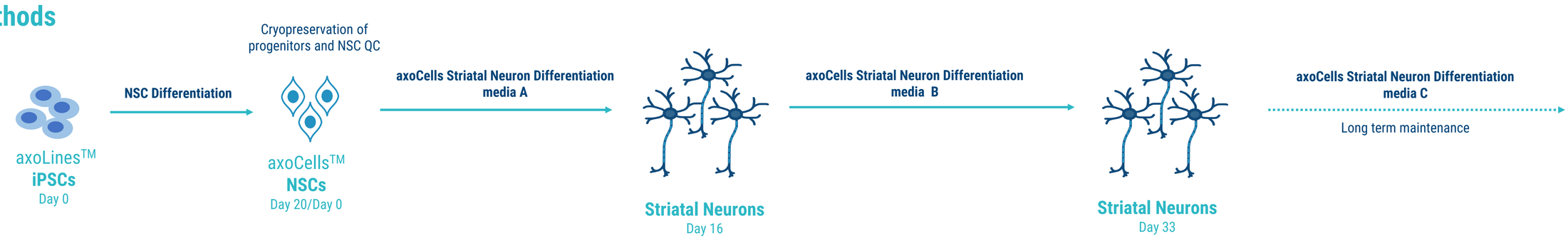
Huntington's Disease (HD) is an autosomal dominant neurodegenerative disorder which has no cure. It is characterized by a profound loss of striatal neurons in the basal ganglia which drives a range of progressive motor, cognitive and psychiatric symptoms. Advanced *in vitro* HD models are a key tool for research and drug discovery, and central to these models is the production of functional striatal neurons[1].

At Axol Bioscience, we have reprogrammed a human induced pluripotent stem cell (iPSC) line from an HD patient with > 120 CAG repeats. After reprogramming and generation of the master bank, the number of CAG repeats was 144 and CAG expansion analysis during 15 passages showed an increase of 1 CAG repeat every 5 passages. Further study suggested that this line has an atypical allele associated with hastening the onset and progression of the disease.

We then differentiated the HD mutant line to produce axoCells™ striatal neurons, together with healthy control-derived neurons. Both cell lines expressed similar levels of key markers including DARPP32, GABA, CTIP2 and MAP2. They also formed neuronal cultures throughout the maturation process, with some morphological differences observed between them; HD striatal neurons exhibited smaller cell bodies and an increased number of neurite branches compared to healthy control neurons, which had larger cell clusters. Furthermore, the line demonstrated more activity compared to the control line upon functional assessment by a multielectrode array system (MEA), 20 days post-maturation.

Further characterization and longitudinal studies of these cell lines are currently ongoing together with the generation of additional HD striatal neurons with different genotypes. This will allow the development of physiologically relevant cellular models with clear disease phenotypes for target identification and drug screening.

Methods



Genetic Characterization

1. Number of CAG repeats increases as our HD cell line expands

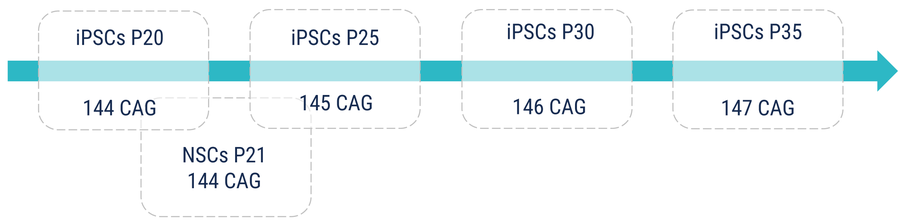


Table 1: Sequence analysis of 3' to CAG repeats

Allele	Sequence
Allele 1	$(CAG)_{18}$ CAACAGCCGCCA(CCG)n
Allele 2	$(CAG)_{138}$ CAGCAGCCGCCG(CCG)n

Table 2: List of SNPs* and INDELS** present in the iPSCs HD > 140 CAG in the HTT gene

Position	Exon	SNP and INDELS
3074921	1	G/A
3074926	1	A/C
3074928	1	CAG/C
3074932	1	AGCAACAG/A
3074945	1	A/G
3107715	Intron 6-7	A/G
3121347	9	C/T
3187820	39	C/A
3214108	50	T/C
3217886	52	A/C
3229934	64	G/A
3240433	67	C/G
3240545	67	C/A
3240580	67	C/T
3241754	67	G/GAAAGGGAGCCCCTGCTC
3241948	A	A/ATTC
3242045	TG	TG/T

*SNP: single nucleotide polymorphism
**INDELS: insertions and deletions

Table 1 and 2: Genetic characterization of mutant allele.

Phenotypic Characterization

Figure 1. Immunofluorescence staining of HD and control-derived axoCells striatal neurons

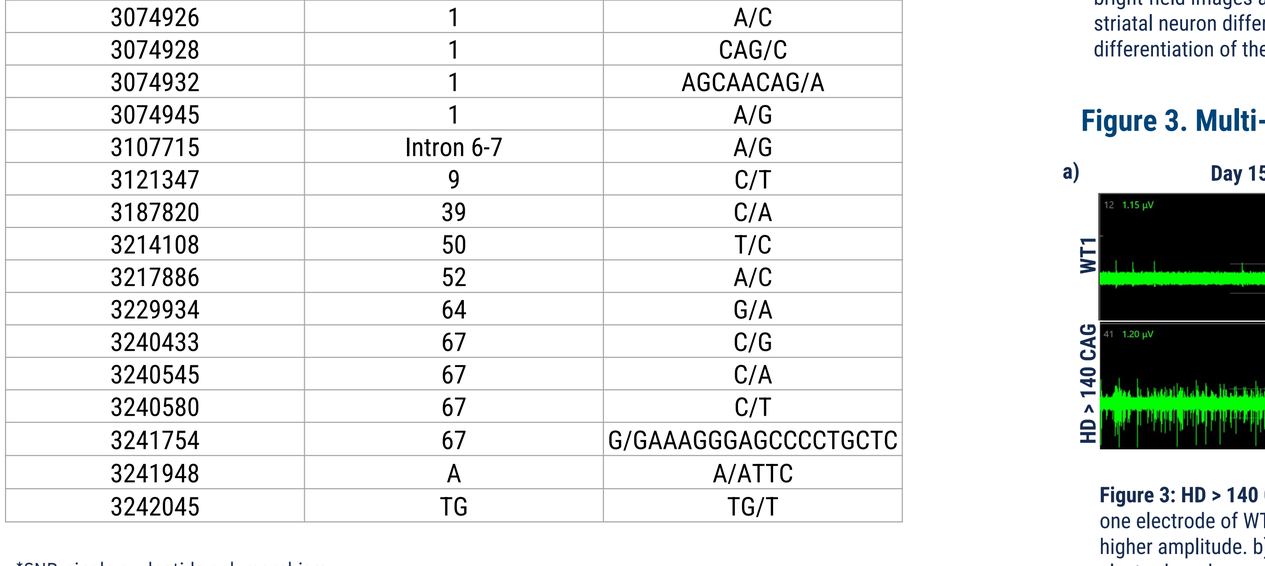


Figure 1: hiPSC HD > 140 CAG striatal neurons express key markers after 31 days differentiation. HD striatal neurons express the markers GABA, CTIP2 and DARPP32, indicative of mature striatal neuron cells, to the same extent as control lines.

Figure 2. Morphology and neurite outgrowth

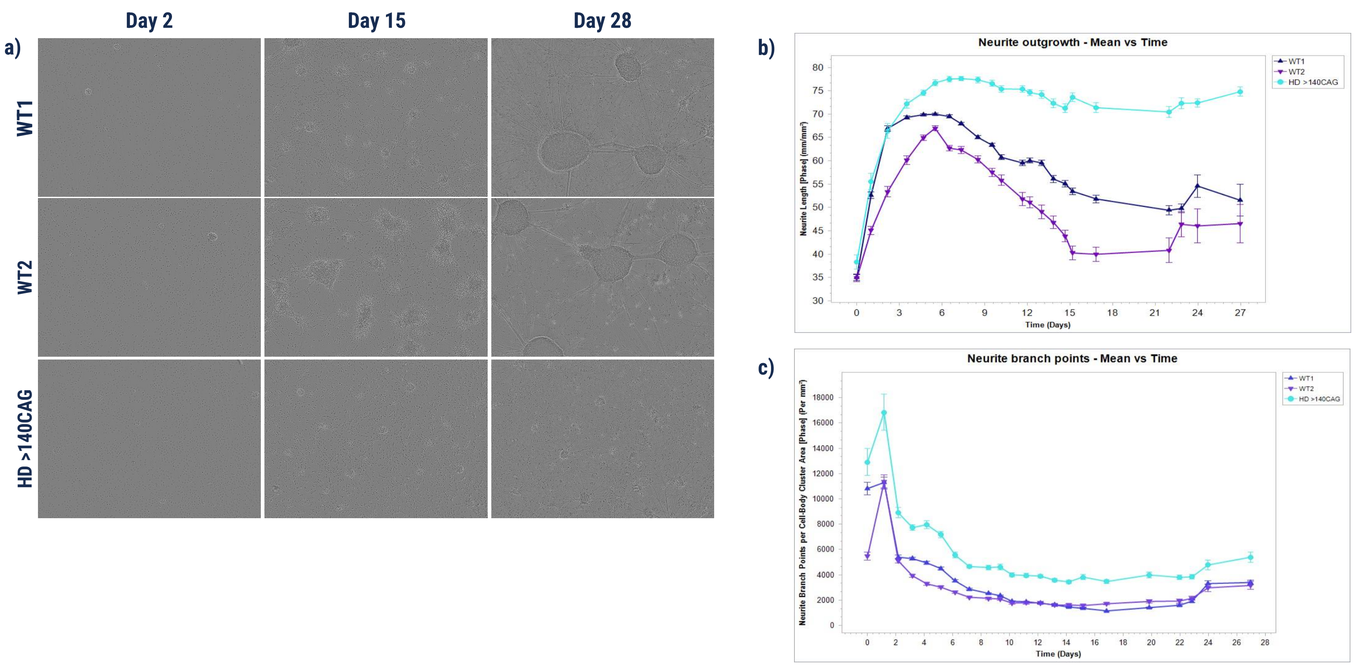


Figure 2: HD > 140 CAG hiPSC line differentiates into striatal neurons as small clusters with a high number of neurite branches. a) Representative bright field images at 4x of healthy control and HD > 140 CAG lines after 2, 15 and 28 days differentiation **b)** Measurement of neurite outgrowth during striatal neuron differentiation of healthy control and HD > 140 CAG lines. **c)** Analysis of neurite branches per cell-body cluster area during the differentiation of the three lines.

Figure 3. Multi-electrode array of HD and control-derived axoCells striatal neurons

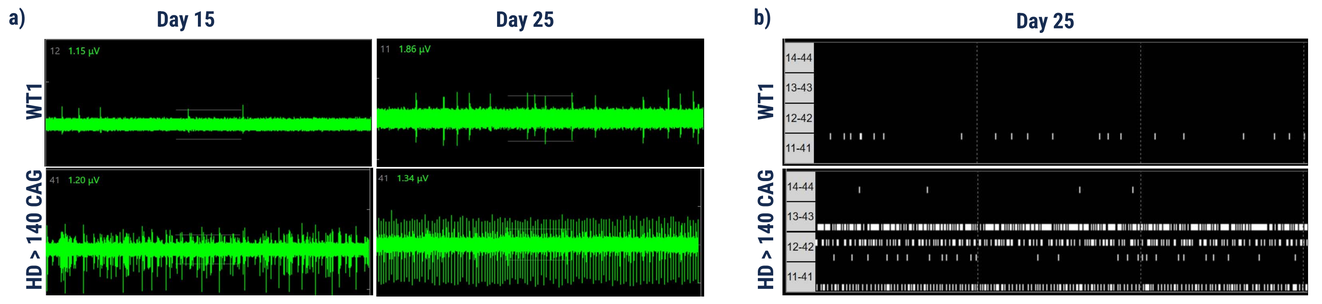


Figure 3: HD > 140 CAG striatal neurons at Day 15 and 25 of differentiation are more active than the control line. a) Representative sodium spikes of one electrode of WT1 and HD striatal neurons at Day 15 and Day 25 of differentiation. The HD line shows earlier activity, more frequent spikes and of higher amplitude. b) Raster plot of 4 different electrodes for each of the lines at Day 25 of differentiation. The mutant line shows activity for most of the electrodes whereas the WT has only active one of the electrodes.

Conclusion

At Axol Bioscience, we have built a human iPSC-derived striatal neuron HD model utilizing a patient line with > 120 CAG repeats. Genetic analysis of the mutant iPSCs highlighted the somatic instability of the CAG repeats sequence which expands every 5 passages, something commonly observed in patients with a high number of CAG expansions. Whole Genome Sequencing (WGS) data of the > 120 CAG iPSC line suggests loss of the CAA interruption and presence of certain SNPs/INDELS in the mutant HTT allele that drive somatic instability, age of onset and HD progression. The HD striatal neurons derived from this line express maturation-associated markers and have a distinct morphological and functional phenotype compared to healthy lines. Further functional longitudinal assessment of the striatal neurons, and the generation of additional cell lines with a range of CAG repeats, will allow us to progress towards target validation and drug screening. Furthermore, we can generate axoCells cortical excitatory neurons, astrocytes and microglia cells from the same HD genetic background to develop complex physiologically relevant co-culture models to better understand and replicate disease phenotypes *in vitro*.

References

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