Characterization of patient iPSCs-derived striatal neurons with >140 CAG repeats over a 100 day-culture

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Abstract

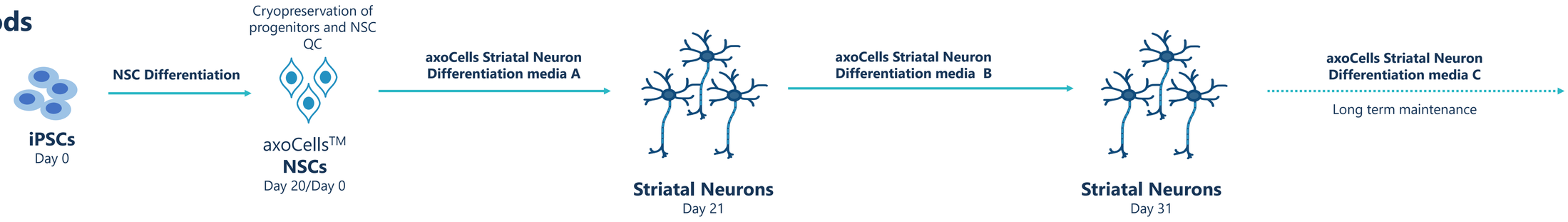
Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder characterised by a profound loss of striatal neurons in the basal ganglia. Patients suffer from a range of motor, cognitive and psychiatric symptoms, with no cure to date. Therefore, the generation of reliable physiologically relevant functional striatal neurons *in vitro* is fundamental to advance in the research of the HD field.

At Axol Bioscience, we have developed human induced pluripotent stem cells (hiPSCs)-derived striatal neurons from healthy individuals and a 125 CAG HD patient. The current bank of HD iPSCs is now 143 CAG repeats. We confirmed that both lines express key striatal neuron markers at RNA and protein level after 20 days in maturation. They form neuronal cultures throughout the maturation process, with some differences observed between HD and control. HD striatal neurons show smaller cellular bodies and an increase in neurite branches compared to WT, which appear with larger cell clusters.

Preliminary data suggests HTT aggregation in the 143CAG HD line when compared to a line with a shorter repeat track. In addition, longitudinal culture of the HD striatal neurons revealed trinucleotide instability, mimicking the somatic instability seen in patients. Functional characterisation by Multi Electrode Array and HTRF for HTT aggregation is ongoing on the 143CAG line and on a patient-derived HD line with 66 CAG repeats. Other patient-derived iPSC lines with different CAG repeat lengths, including 39, 42, and 38 CAGs (unaffected at time of sampling), have also been matured to striatal neurons.

Over the past year, we've worked on the generation of a gene edited WT isogenic control for the 143CAG line and, during this process, a 47 CAG repeats isogenic line was also identified. Efforts are ongoing to complete full characterisation of these lines, before making them available to the HD research community to continue to support the development of physiologically relevant cellular models with clear disease phenotypes for target identification and drug screening.

Methods



1. CAG instability on 143 CAG HD line

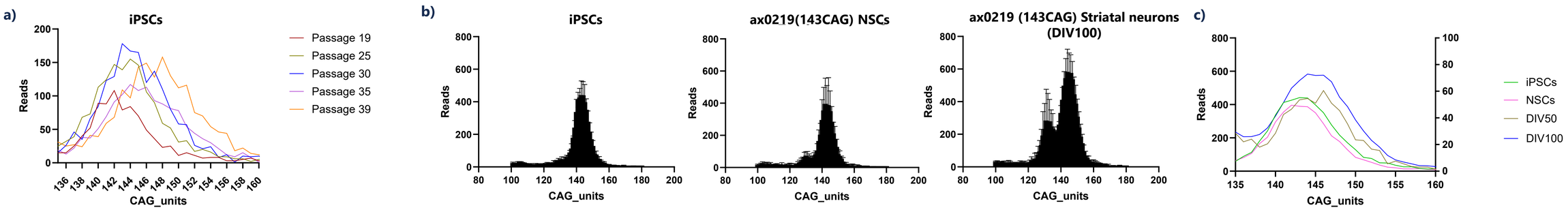


Figure1: (a) The CAG repeat track of the 143 CAG repeat HD iPSC line at increasing passages was sequenced using Oxford Nanopore technology, and this revealed a progressive expansion of the CAG repeats in the mutant allele as the cells replicate in culture. Fragment analysis confirmed that the there is an increase of 1X CAG repeat every 5 passages (0.4X CAG/week) under Axol's standard cell culture conditions (data not shown). **(b)** When the iPSCs differentiate to striatal neurons, the CAG repeats expand at a rate of 0.13X CAG/week. Striatal neurons are terminally differentiated cells that do not proliferate in culture. **(c)** Longitudinal culture of the HD striatal neurons revealed trinucleotide instability, mimicking the somatic instability seen in patients.

2. Phenotypic characterisation of ax0219 striatal neurons

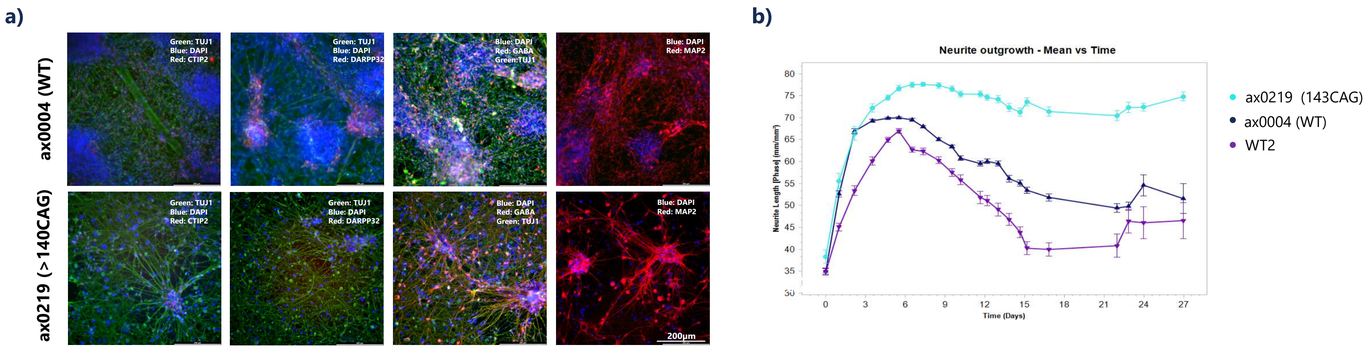


Figure 2: (a) ax0219 striatal neurons express mature striatal neuron markers MAP2, CTIP2, GABA, DARPP32. **(b)** HD 143 CAG hiPSC line differentiates into striatal neurons as small clusters with a high number of neurite branches, quantified by neurite outgrowth.

3. Quantification of aggregation and mHTT of ax0219 striatal neurons

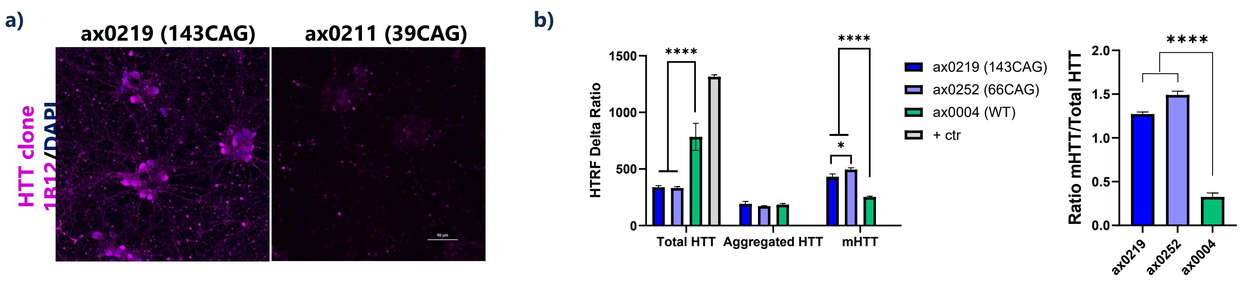


Figure 3: (a) Staining of ax0219 (143CAG) and ax0211 (39CAG) HD lines with anti HTT NEO P90 antibody clone 1B12 shows stronger signal and presence of more aggregates in ax0219 striatal neurons compared to a line with a shorter CAG track. **(b)** HTRF quantification of total, aggregated and mutant HTT protein confirmed more mutant HTT in HD striatal neurons (n=3 technical replicates).

4. Quantification of electrical activity of ax0219 striatal neurons

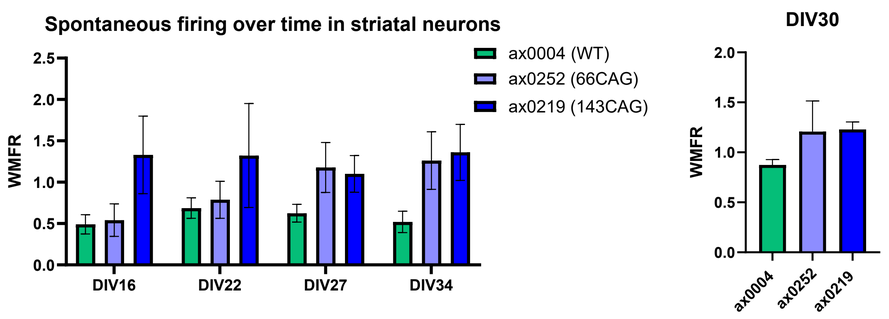


Figure 4: ax0219 HD striatal neurons are more electrically active than the control line, assessed by spontaneous firing (weighted mean firing rate, WMFR) on MEA.

5. Transcriptomics of ax0219 striatal neurons

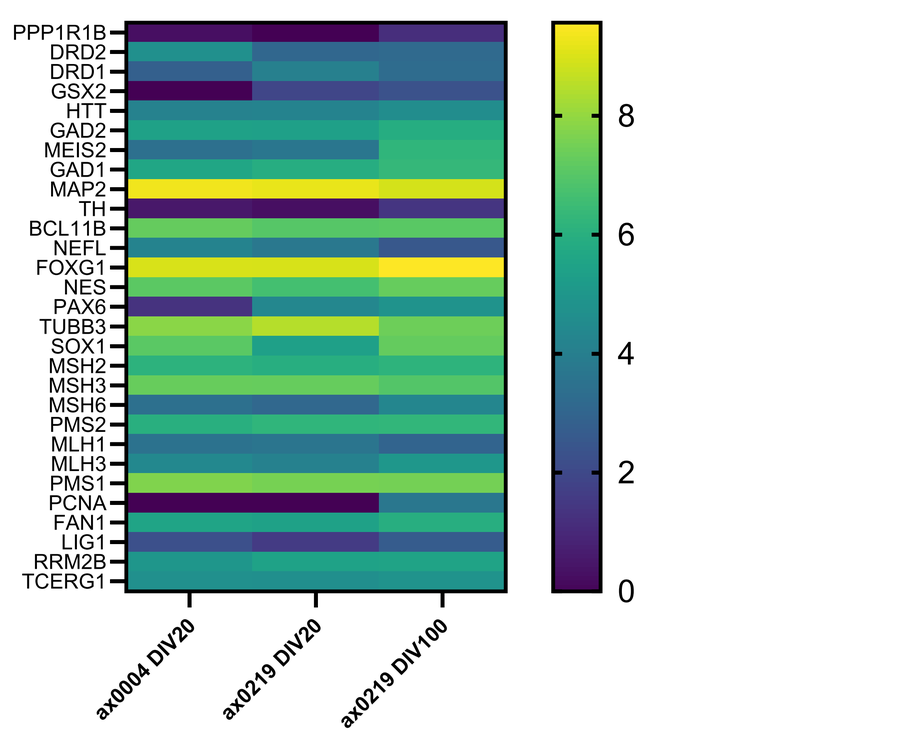


Figure 5: Heatmap showing relative expression levels of striatal neurons, NSCs and MMR genes measured by TempO-Seq (BioClavis) across ax0004 (WT) at DIV20 and ax0219 (143CAG) at DIV20 and DIV100. Values represent the mean of three technical replicates per sample. Expression values were normalised prior to analysis. Colour intensity indicates relative expression levels as shown in the scale bar.

6. Other Axol hiPSC lines with different CAG sizes can differentiate to striatal neurons

Cell line name	Disease Status	Sex	Age	Gene	Confirmed Genetic Mutation
CENSOi011-D	Affected	F	45-49	HTT	CAG18/CAG39
CENSOi017-A	Affected	F	51	HTT	CAG17/CAG42
CENSOi019-A	Affected	F	7	HTT	CAG14/CAG125 (now CAG134)
CENSOi019-B	Affected	F	7	HTT	CAG14/CAG125 (now CAG143)
CENSOi052-A	Affected	M	16	HTT	CAG28/CAG66
CENSOi053-A	Asymptomatic Carrier	M	64	HTT	CAG17/CAG38

Conclusion

At Axol Bioscience, we have built a human iPSC-derived striatal neuron HD model utilizing a patient line with 143 CAG repeats (125 at sampling). 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. Terminally differentiated striatal neurons derived from this line expand at a slower rate of 0.13CAG/week. The line have a distinct morphological and functional phenotype, including more mHTT and higher electrical firing rate compared to healthy lines. Maturation-associated markers are expressed after 21 days in maturation and remain constant for 70 more days. Also, we are generating a WT isogenic control line for ax0219, that together with further characterization of striatal neurons of additional cell lines with a range of CAG repeats will help us build a comprehensive platform to use towards target validation and drug screening. Moreover, 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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