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

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Abstract

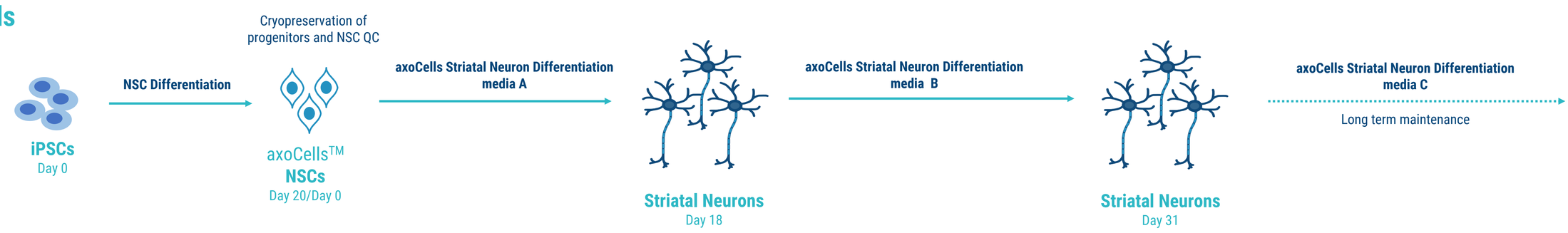
Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder characterized by a profound loss of striatal neurons in the basal ganglia. Patients suffer from a range of motor, cognitive and psychiatric symptoms, with no cure to date. Therefore, the generation of reliable physiologically 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 because of its time in culture. We confirmed that both lines express key striatal neuron markers at RNA and protein level after 20 days in maturation.

In collaboration with Harness Therapeutics, we observed that both cell lines formed neuronal cultures throughout the maturation process, with some differences observed between HD and control. HD striatal neurons showed smaller cellular bodies and an increase in neurite branches compared to wildtype (WT), which appeared with larger cell clusters. In addition, longitudinal culture of the HD striatal neurons revealed trinucleotide instability, mimicking the somatic instability seen in patients. Functionally, HD striatal neurons are more active than control neurons when assessed by Multi Electrode Array (MEA).

Other patient-derived iPSC lines with different CAG repeat lengths, including 39 CAG, 42 CAG, 38 CAG (unaffected at time of sampling) and 66 CAG, have also been matured to striatal neurons. Further characterization and longitudinal studies of these cell lines are currently ongoing. This will allow the development of physiologically relevant cellular models with clear disease phenotypes for target identification and drug screening.

Methods



1. Genetic Characterization of HD hiPSCs with >140 CAG

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

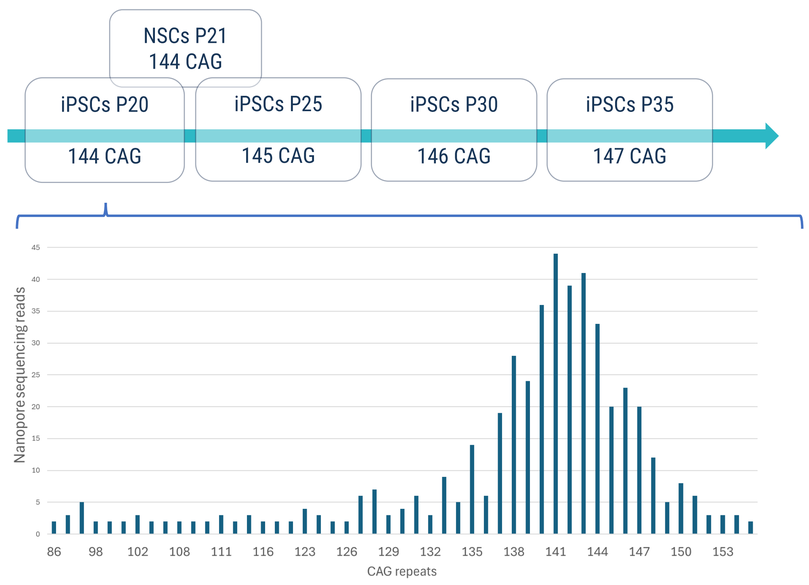


Figure1: HD iPSCs expand 1X CAG repeat every 5 passages (0.4X CAG/week) under Axol's standard cell culture conditions. Distribution of the number of the CAG repeats of the mutant allele in the population is confirmed by Nanopore sequencing.

Table 1: List of SNPs* and INDELS** present in the iPSCs HD > 140CAG

Position	Exon	SNP and INDELS
3074613	5'UTR	$(GGCCCCGCCTCCGCCGGCGC)_3$
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: Genetic characterization of mutant allele.

3. 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	40-50	HTT	CAG18/CAG39
CENSOi017-A	Affected	F	51	HTT	CAG17/CAG42
CENSOi019-A	Affected	F	7	HTT	CAG14/CAG127 (now CAG134)
CENSOi019-B	Affected	F	7	HTT	CAG14/CAG127 (now CAG143)
CENSOi052-A	Affected	M	16	HTT	CAG28/CAG66
CENSOi053-A	Asymptomatic Carrier	M	64	HTT	CAG17/CAG38

2. HD hiPSCs-derived striatal neurons with >140 CAG can mature for 100 days

1. HD striatal neurons >140 CAG phenotypic characterisation after 31 days in maturation

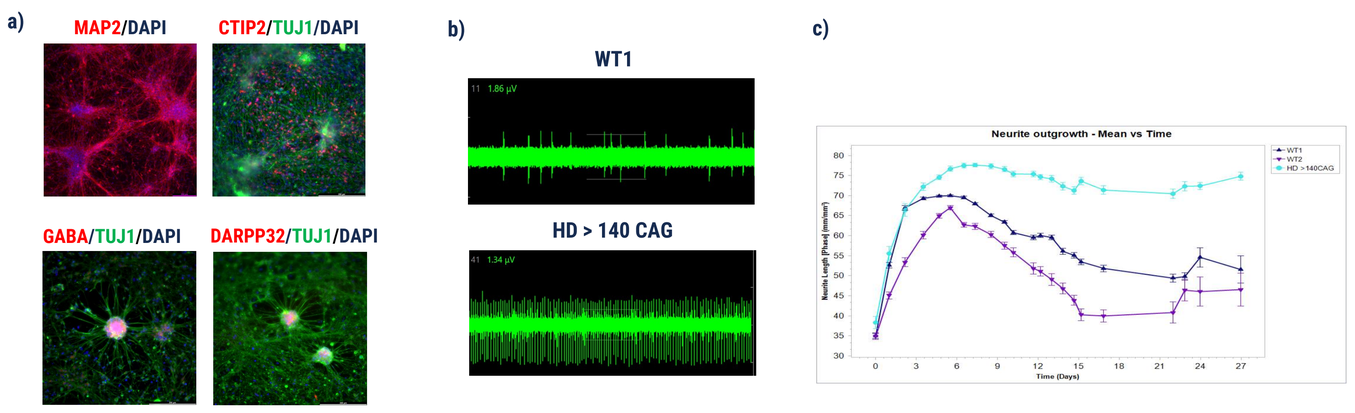


Figure 2: (a) HD striatal neurons express the markers MAP2, CTIP2, GABA, DARPP32, indicative of mature striatal neuron cells. **(b)** HD > 140 CAG striatal neurons at Day 25 of differentiation are more active than the control line. Representative sodium spikes of one electrode of WT1 and HD striatal neurons show more frequent spikes and of higher amplitude. **(c)** HD > 140 CAG hiPSC line differentiates into striatal neurons as small clusters with a high number of neurite branches, quantified by neurite outgrowth.

2. Optimisation of anti HTT NEO P90 antibodies in HD striatal neurons

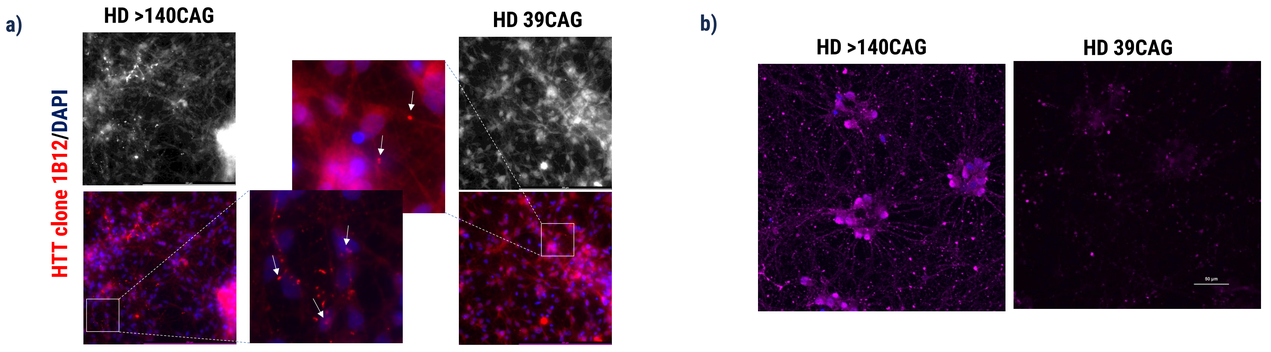


Figure 3: Optimisation of anti HTT NEO P90 antibodies clone 1B12 **(a)** and high magnification images acquired by Nikon of the same cell lines and antibody **(b).**

3. HD striatal neurons longitudinal studies for up to 100 days

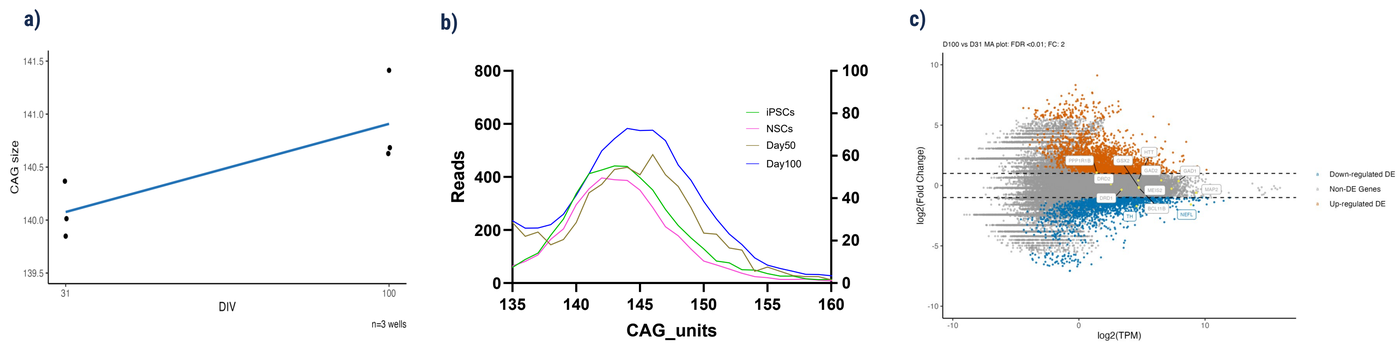


Figure 4: (a) HD Striatal neurons expands at slower rate than iPSCs (0.13X CAG/week) when assessed during a 100 day-maturation. The number of CAG repeats was quantified using Nanopore sequencing data after quality control steps. The weighted arithmetic mean of the CAG distribution was then calculated to determine the CAG size for each sample. **(b)** The number of uninterrupted CAG repeats was quantified using Nanopore sequencing after PCR amplification. **(c)** Bulk RNA-seq analysis with paired-end 150 bp sequencing reads aligned against human genome (hg38) with HISAT2 of HD striatal neurons after 31 days and 100 days in maturation show that key markers expression remains constant. The biological coefficient of variation (BCV), was set to 0.2 due to the absence of biological replicates. DE genes were defined with Benjamini-Hochberg adjusted P < 0.01 and fold change ratio > 2.

4. Conclusion

At Axol Bioscience, we have built a human iPSC-derived striatal neuron HD model utilizing a patient line with > 140 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 >140CAG iPSC line suggests loss of the CAA interruption after the CAG repeats and presence of certain SNPs/INDELS in the mHTT allele that drive somatic instability, age of onset and HD progression. The HD striatal neurons derived from this line have a distinct morphological and functional phenotype compared to healthy lines. Maturation-associated markers are expressed after 31 days in maturation and remain constant for 70 more days, and the repeat region expand 1 extra CAG repeat during that time. 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. 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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