PATIENT-DERIVED hiPSCs AS A RESOURCE FOR MODELLING HUNTINGTON'S DISEASE

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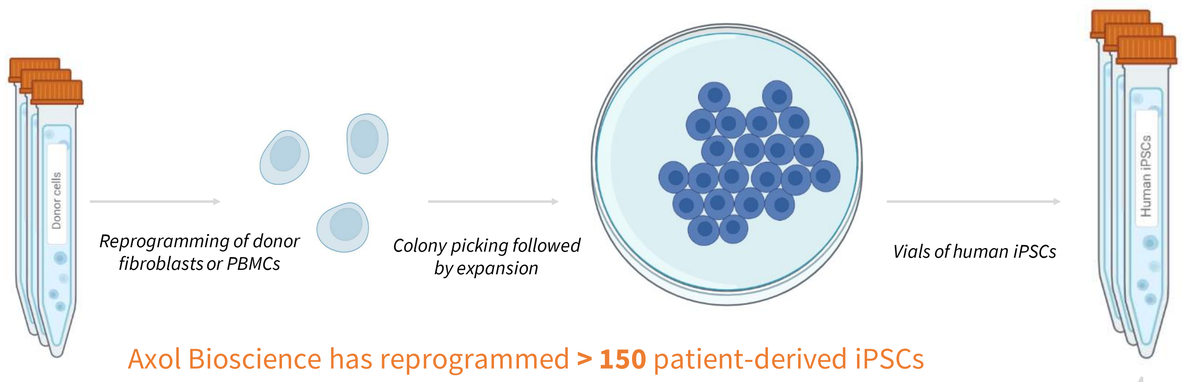
Abstract

Huntington's disease (HD) is an autosomal dominant neurodegenerative condition caused by repeat expansions of CAG trinucleotides in the huntingtin gene. The development of animal models for HD in a range of species has provided insights into its pathogenesis and allowed the generation of potential therapies. However, these showed limited or no efficacy when tested in clinical trials. Thus, there is an unmet need for physiologically relevant human cellular platforms for in vitro drug screening.

At Axol Bioscience Ltd, we utilise our expertise on reprogramming, CRISPR/Cas9 genome editing, and disease-relevant cell differentiations to generate hiPSCs-derived in vitro models. We have reprogrammed >150 patient-derived iPSC lines, from which 46 are derived from HD donors spanning 39 to 127 CAG trinucleotide repeats using the Sendai-virus based CytoTune 2.0 kit. The hiPSC lines have been qualified based on a range of tests, including microbiology, growth profile, cell line identity, vector clearance, karyology, pluripotency assessment and differentiation potential to ensure they are of the highest standards. We have also successfully differentiated 2 independent clones of HTT >120CAG to microglial cells, cortical neurons and astrocytes highlighting the potential of these lines to become HD-relevant cell types. This, together with the development of isogenic controls, constitutes a powerful platform for high-content screenings and developing gene therapy.

Human iPSC Production Process



Axol Bioscience has reprogrammed **> 150** patient-derived iPSCs

Quality Control Test	Method	Standard/Optional
Microbiology	Sterility by broth	Standard
	Mycoplasma PCR/qPCR	Standard
	Viral screen of primary cells	Standard
Viability/Growth profile	Visual record/Images	Standard
	Record of growth	Standard
Cell line identity	Genetic lesion	Optional (recommended)
	STR	Standard
Clearance of reprogramming factors	qPCR analysis	Optional (recommended)
Karyology	G-Banding	Optional
	aGH analysis	Optional
Pluripotency-associated marker assessment	Flow cytometric analysis	Optional
Differentiation potential	EB Scorecard	Optional

HD modelling with iPSCs and CRISPR-Cas9 technology

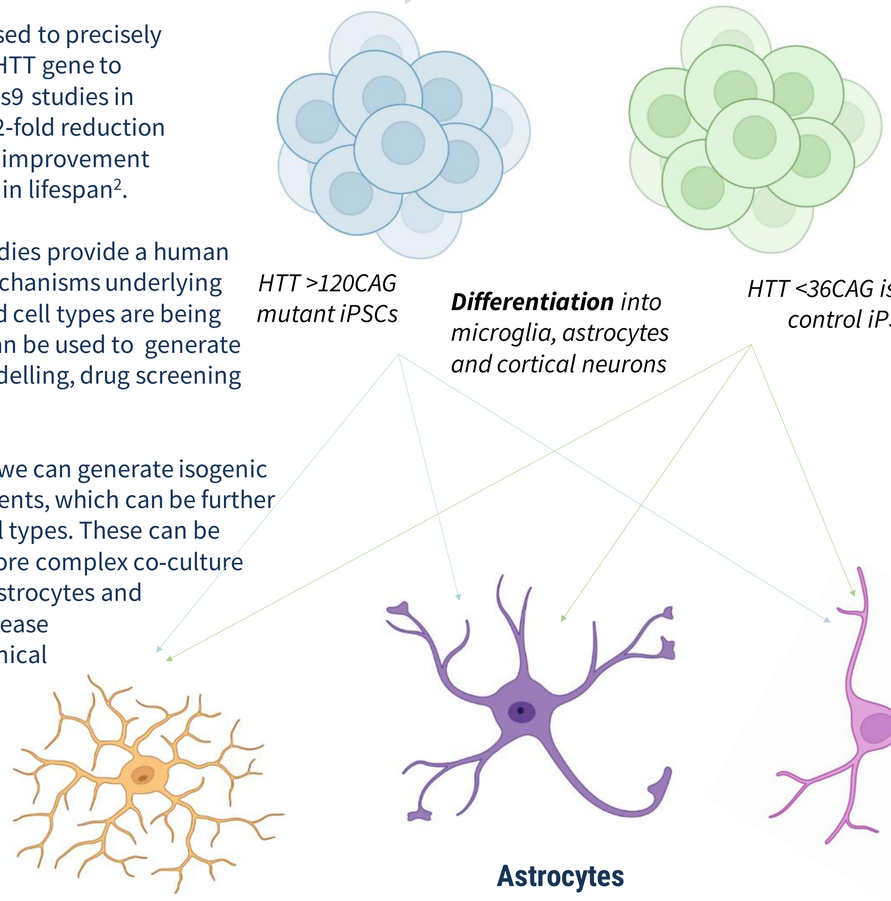
HD is a rare, progressive neurodegenerative disease caused by expansion of CAG trinucleotide repeat in exon1, which results in abnormal protein folding and formation of aggregates ultimately disrupting important cellular functions in the brain. Although there are therapies available (such as neurotransmitters and antipsychotics) that help manage and alleviate symptoms of HD, there is currently no cure for HD.

The Huntingtin (HTT) protein is ubiquitously expressed in tissues and cells, and covers diverse cellular roles including, apoptosis, vesicle transport, cell signalling and transcriptional regulation[1]. The exact mechanisms by which mutant HTT aggregates destroy neurons is yet to be fully understood. However, several years of research in the field using cell lines and animal models indicates that reducing the expression of the mutant HTT protein might halt progression of the HD.

Genome editing technologies can be used to precisely alter the DNA sequence of the mutant HTT gene to reduce the CAG expansions. CRISPR-Cas9 studies in mice to alter mutant HTT have shown 2-fold reduction in formation of neurotoxic aggregates, improvement in motor neuron function and increase in lifespan[2].

Pluripotent stem cells (PSC) based studies provide a human cell relevant model to understand mechanisms underlying complex disorders in-vitro. PSC derived cell types are being used extensively in the field as these can be used to generate large quantities of cells for disease modelling, drug screening and ultimately cell therapy.

By using the CRISPR-Cas9 technology, we can generate isogenic pairs of PSC lines derived from HD patients, which can be further differentiated to different neuronal cell types. These can be used either in 2D monoculture or in more complex co-culture models, combining Striatal Neurons, Astrocytes and Cortical Neurons to understand the disease mechanism, phenotype and for pre-clinical validation of targets.



Assay characterisation of differentiated cells

Microglia

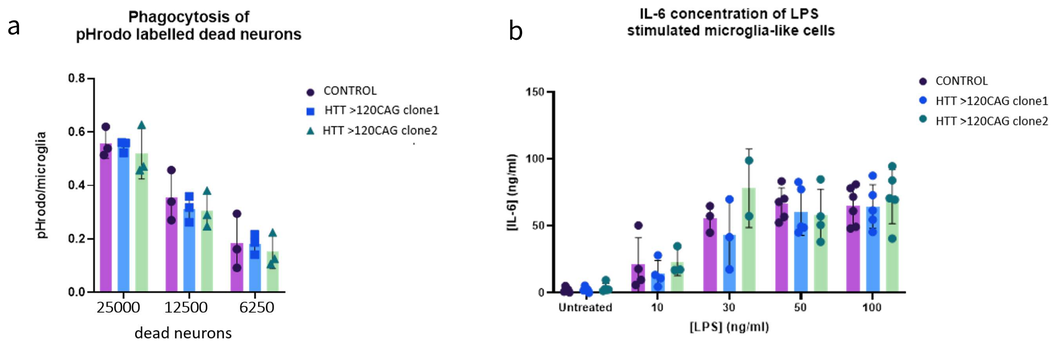


Figure 4: (a) Effect of decreasing concentrations of dead neuron bait on iPSCs-derived control and HD microglial phagocytosis. (b) Effect of increasing concentrations of LPS on IL-6 release from microglia.

Astrocytes

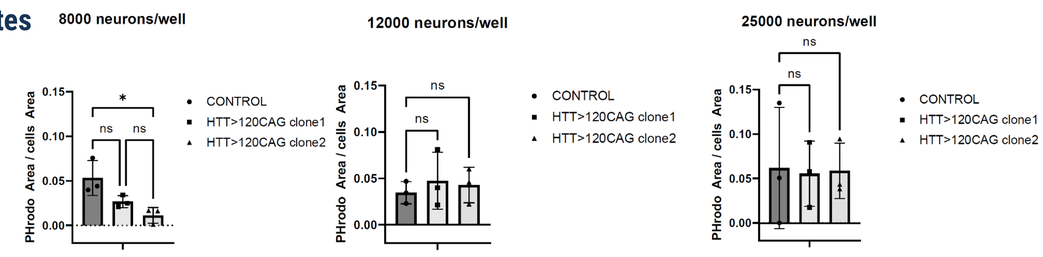


Figure 5: Comparison of phagocytosis on iPSCs-derived control and HD astrocytes with varying numbers of pHrodo-labelled dead neurons as bait.

Neural stem cells

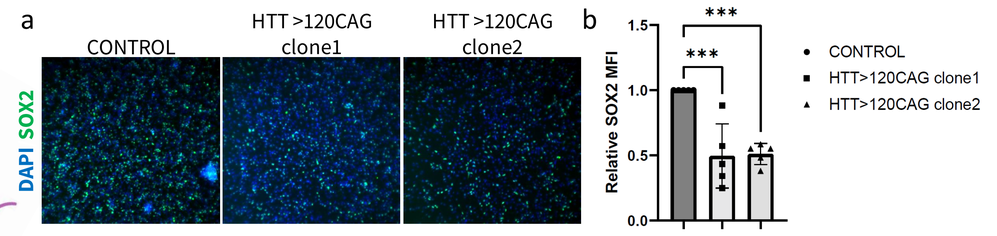


Figure 6: SOX2 expression assessment by immunocytochemistry in iPSCs-derived control and HD neural stem cells (a) and its quantification (b).

Marker expression of differentiated cells

Microglia

DAPI P2Y12 DAPI IBA1 DAPI CX3CR1 DAPI TMEM119

Figure 1: Representative immunocytochemistry images of microglial markers in iPSCs-derived control and HD microglial cells. Column 1 and 2 were acquired at 20x magnification. Columns 3 and 4 were acquired at 40x.

Astrocytes

DAPI ALDH1 S100 DAPI A2B5 EAAT1 DAPI NESTIN GFAP

Figure 2: Representative immunocytochemistry images of astrocyte markers in iPSCs-derived control and HD astrocytes. 20x magnification.

Cortical neurons

DAPI TUJ1 DAPI MAP2 DAPI GLS1

Figure 3: Representative immunocytochemistry images of cortical neuronal markers in iPSCs-derived control and HD cortical neurons. 20x magnification.

References

1. Anglada-Huguet M, Vidal-Sancho L, Cabezas-Llobet N, et al. (2017) Pathogenesis of Huntington's Disease: How to Fight Excitotoxicity and Transcriptional Dysregulation. Huntington's Disease - Molecular Pathogenesis and Current Models. InTech. DOI: 10.5772/66734.
2. Ekman FK, Ojala DS, Adil MM, et al. (2019) CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model. Molecular Therapy – Nucleic Acids. DOI: //doi.org/10.1016/j.omtn.2019.07.009

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