Modelling neurodegeneration using a human isogenic system: A next generation approach to study Huntington's disease

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Abstract

The development of therapies to treat patients with neuronal indications is currently hampered by the use of animal models, as less than 10% of findings derived from these preclinical models can be translated to humans. Patient-derived induced pluripotent stem cells (iPSCs) offer the possibility to generate in vitro systems to model neurological diseases that can recapitulate relevant human disease phenotypes. However, conventional human iPSC (hiPSC) differentiation protocols are often lengthy, inconsistent, and difficult to scale. More importantly, the lack of genetically matched controls for patient-derived models further complicates the investigation of disease-relevant phenotypes and study of molecular mechanisms underlying neurodegeneration.

To overcome these problems, we developed a proprietary gene-targeting strategy (opti-ox™) that enables highly controlled expression of transcription factors to rapidly reprogram hiPSCs into any specific somatic cell type in a scalable manner. Our reprogramming approach together with CRISPR/Cas9-mediated genetic engineering enables us to introduce specific mutations in these hiPSC lines and create isogenic disease models that will improve screen specificity and accelerate drug development.

We used our opti-ox induced ioGlutamatergic Neurons to generate a Huntington's disease (HD) model that carries a heterozygous 50 CAG trinucleotide expansion in the huntingtin (*HTT*) gene. Mutant HTT proteins containing elongated polyglutamine (PolyQ) stretches are aggregation-prone and have been reported to affect a range of neuronal subtypes, including cortical glutamatergic neurons. Here we present the gene and protein expression profile of the ioGlutamatergic Neurons HTT $^{50CAG/WT}$ (disease model) in comparison to the wild type ioGlutamatergic Neurons (isogenic control), and further phenotypic characterisation data showing differences in their transcriptome and neuronal activity.

1. Generation of a 50 CAG trinucleotide repeat expansion in the huntingtin gene

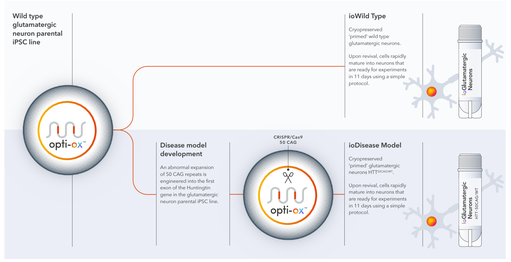
A heterozygous 50 CAG mutation in the *HTT* gene was introduced into a wild type ioGlutamatergic Neuron cell line using CRISPR/Cas9 gene editing to generate an isogenic disease model.

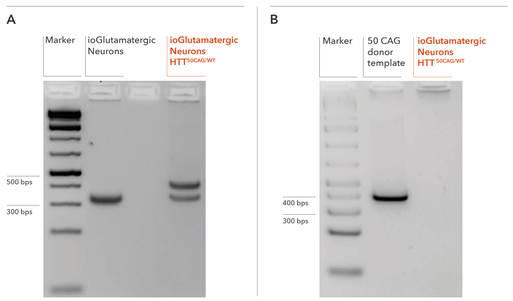
Successful on-target integration into one *HTT* allele confirmed by gel electrophoresis.

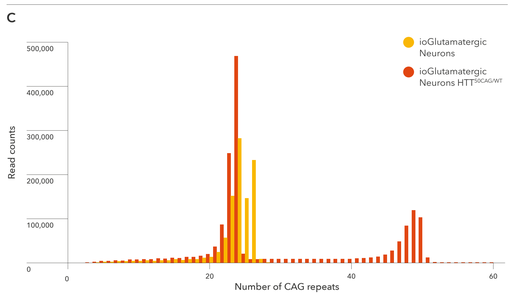
(A) Genotyping primers flanking the endogenous *HTT* CAG repeat expansion region produce a band at approximately 320 base pairs (bps) by PCR, in both isogenic control and disease model. PCR fragments at 395 bps detect on-target gene editing and introduction of a 50 CAG repeat expansion in the disease model only. (B) Amplicon PCR of the plasmid donor reveals no random integration in genomic DNA from targeted colonies via gel electrophoresis. Off-target random insertion of the donor template (used to introduce the 50 CAG repeat expansion at the wild type *HTT* locus) is detected by PCR amplification of the donor vector backbone. This is not detected in the samples from the disease model.

NGS-Amplicon sequencing.

(C) NGS-amplicon sequencing confirms the number of CAG repeats in isogenic control (yellow) and disease model (orange). The number of CAG repeats shows a peak at the normal physiological range of 24 for both the wild type and disease model cells. The 50 CAG repeat was detected only in the mutant cells (orange) confirming the successful introduction of a heterozygous 50 CAG repeat expansion.







2. Characterisation of the Huntington's disease model

A. Formation of structural neuronal networks over 11 days.

Neuronal cultures of both isogenic control and disease model mature at identical rates forming more complex neuronal network structures over time.

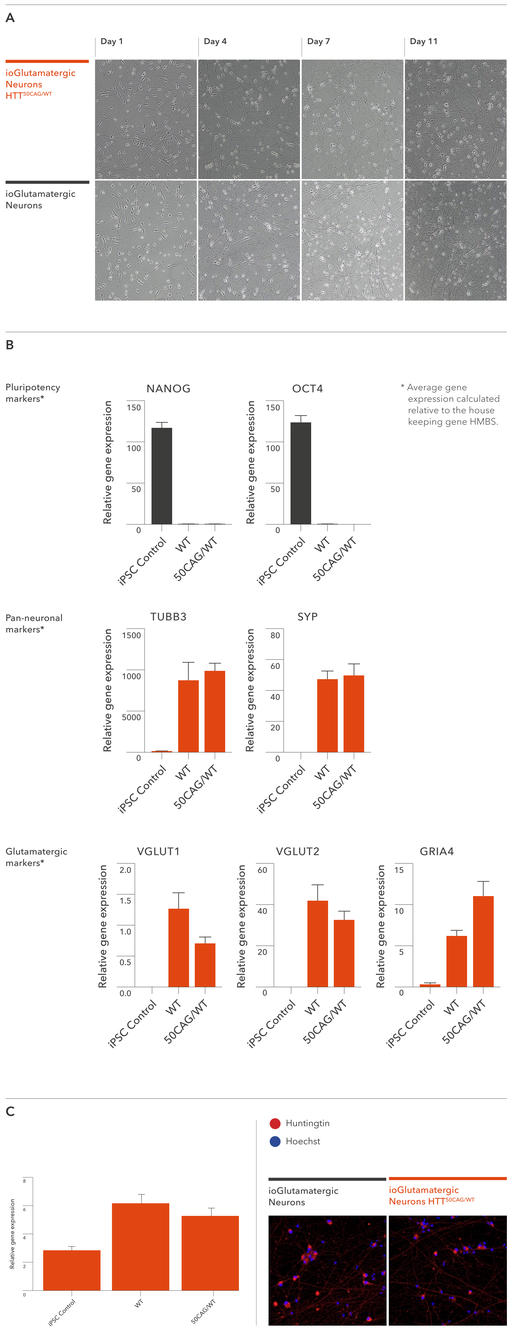
B. Disease model cells and isogenic control have highly similar gene expression patterns.

RT-qPCR analysis was performed on cultures of the isogenic control and disease model at day 11. cDNA samples of the parental iPSC line were included as reference.

Both cultures lack the expression of pluripotency markers (NANOG and OCT4), while robustly expressing pan-neuronal (TUBB3 and SYP) and glutamatergic-specific (VGLUT1 and VGLUT2) genes, as well as the glutamate receptor GRIA4.

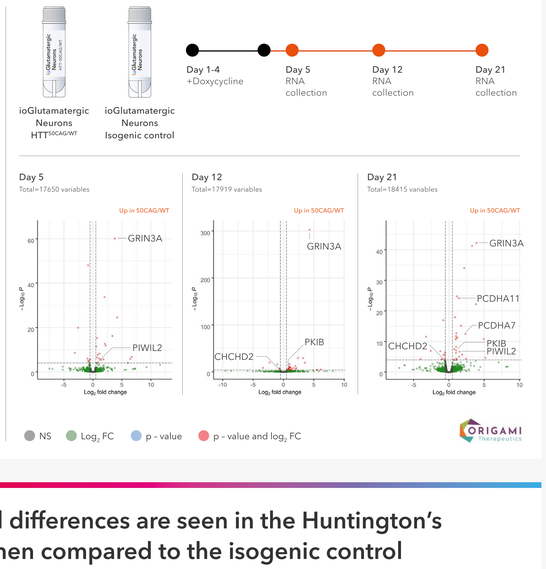
C. Huntingtin expression in the disease model cells and their isogenic control.

Huntingtin was expressed in both isogenic control and disease model cells at day 11 as assessed by RT-qPCR (left panel) and immunocytochemistry (ICC) analysis at day 20 post-revival (right panel; red= HTT; blue= Hoechst). ICC images provided courtesy of Origami Therapeutics and Scintillant Bioscience.



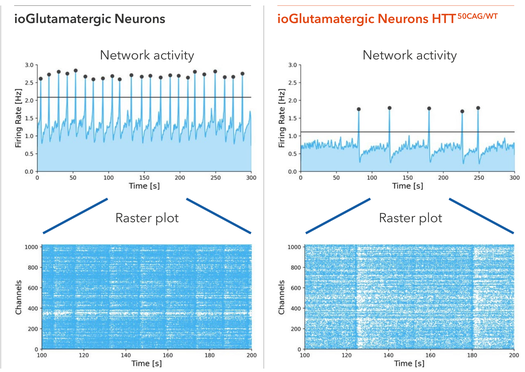
3. Bulk RNA-seq analysis reveals differentially expressed genes in the Huntington's disease model

Bulk RNA-seq was used to compare the disease model cells with the isogenic control. Four replicates of the experiment were run, taking RNA samples at three time points, days 5, 12 and 21. *GRIN3A* (glutamate receptor NMDA type subunit 3A) was seen to be expressed at higher levels in the disease model cells. This marker has previously been detected in the striatum of HD patients (Marco et al., 2013). Our data also suggest a dysregulation of protocadherins (*PCDHA7, PCDHA11*) as has been previously reported for a HD mouse model (Langfelder et al, 2016). *CHCHD2* is seen to be down regulated in the disease model cells. This gene is involved in mitochondrial respiration and is a genetic risk factor for several neurodegenerative disorders. The abnormal expression of these genes could indicate interesting cellular phenotypes relevant for Huntington's disease. Data provided by Origami Therapeutics.



4. Electrophysiological differences are seen in the Huntington's disease model cells when compared to the isogenic control

In collaboration with Charles River Laboratories, we used the MaxTwo high-density microelectrode array (MaxWell Biosystems) to compare the network activity of the isogenic control and disease model cells. We performed a Network Assay to record synchronous activity at day 38. The firing rate is displayed graphically (above) and as a Raster plot (below), where the number of active electrodes (blue) over time are shown, with a vertical blue line indicating synchronised activity. The data show that compared to the isogenic control, the disease model has a reduced network activity, as has been reported previously for iPSC-derived HD models (Metha et al. 2018).



Summary & conclusions

Using CRISPR/Cas9 editing we successfully introduced a 50 CAG trinucleotide expansion in exon 1 of the *HTT* gene in an opti-ox enabled ioGlutamatergic Neuron cell line creating a novel isogenic human Huntington's disease model.

Characterisation of the ioGlutamatergic Neurons HTT $^{50CAG/WT}$ cells demonstrated similar morphology, gene and protein expression profiles to the isogenic control. Interestingly, we identified several differentially expressed genes that could be relevant for HD, including *GRIN3A*, protocadherins and *CHCHD2*, and detected altered functional characteristics in our HD cell model relating to a reduced neuronal firing rate and network activity.

ioGlutamatergic Neurons HTT $^{50CAG/WT}$ represents a robust and scalable human iPSC-derived Huntingdon's disease model with a physiologically relevant phenotype. Combined with ioGlutamatergic Neurons, the wild type genetically matched control, the disease model is suitable for translational research and high-throughput drug discovery applications.