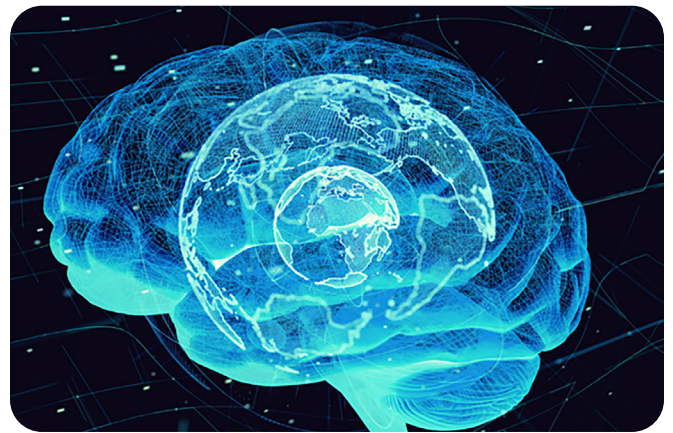


Utility of engineered human Neural Stem Cells (NSCs) expressing varying exon 1 HTT fragments to study Huntington's disease

Introduction

Huntington's disease (HD) is a rare, progressive neurodegenerative disorder that usually presents itself with symptoms of motor and cognitive decline between the ages of 30 and 50. It is most commonly caused by an expansion mutation in the CAG trinucleotide repeat within exon 1 of the HTT gene which codes for the protein huntingtin and is inherited in an autosomal dominant manner. There is currently no cure and most treatment strategies aim to slow down the progression and treat specific symptoms to help with quality of life as much as possible.

Research in human and different animal models, such as mouse and drosophila, plus cell systems has tied the length of the CAG repeat that translates into polyglutamine (PolyQ) stretches within the N-terminal domain of the protein with not only disease of penetrance, but attribute this "exon 1 fragment" mutation as a driver of neurotoxicity in HD that leads to neuronal death responsible for much of the symptoms. Biologically relevant cellular models are key to not only better understanding the biological processes involved in HD disease modeling of these CAG expansions, but also for use in pre-clinical screening applications to help accelerate disease research and drug discovery. To this end, Dr. Ghosh and Dr. Tabrizi's team engineered a human neural stem cell model based on ReNcell VM cultures that stably expressed exon 1 HTT fragments of varying polyQ-length expansions that easily differentiated into mostly midbrain GABAergic neurons while maintaining exogenous expression of exon 1 HTT fragments; this cell culture system also displayed early phenotypes of HD in some cells, such as the formation of inclusion bodies and mitochondrial dysfunction,



which could be used to assess metrics of therapeutic efficacy of drugs in a high-throughput manner coupled as with high content analysis.

For the generation of this novel HD model cell system, ReNcell VM cell cultures were transduced with lentiviral particles containing vectors that included HTT exon 1 with either 30, 71 or 122 CAG repeats linked with an internal ribosome entry site (IRES) to GFP. The 122 CAG stretch contained a CAA interruption, and cells containing the different repeat lengths with similar expression pattern were selected for use in the studies with the necessary controls. Ultimately, as each CAG repeat sequence was followed by CAA CAG codons, their cell panels included those expressing low expression levels of 32Q, 73Q, and 124Q exon 1 HTT.

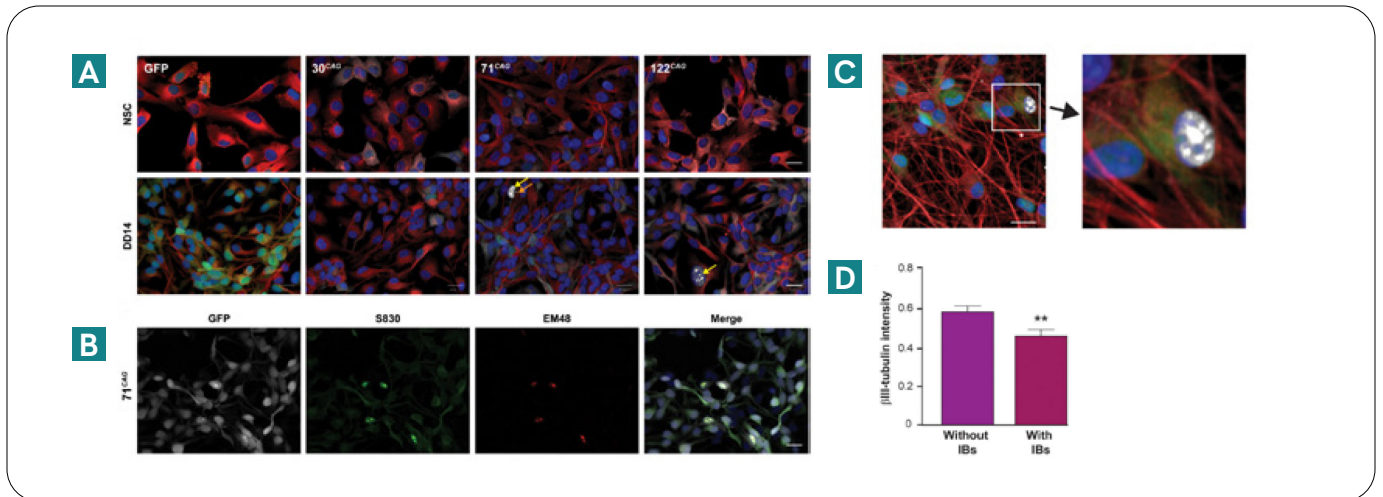


Figure 1: Taken from Figure 2, exploring appearance of inclusions in the 71 and 122 CAG transfected cells compared to negative or 30 CGG transfected cells at 14 days post initiation of differentiation and culture along with other markers using immunofluorescence imaging and confirmed via western blot analysis. Additional method and image details can be found in the method and figure legend of doi.org/10.1096/fj.201902277RR.

Subsequent characterization showed the expression of neuronal marker β III-tubulin protein levels were similarly expressed upon differentiation by immunofluorescence and western blot. Prior to differentiation to neuronal cultures (DD14), no extra- or intra nuclear HTT inclusions were observed across the panel; however, the 71 CAG and 122 CAG lines showed intranuclear IBs and a small number of extra-cellular IBs by immunofluorescence that also stained positive for anti-aggregated HTT antibodies S830 and EM48, as well as PHP1, and PHP2 anti-HTT antibodies, which correlated with lower or absent β III-tubulin perinuclear cytoplasmic staining in those regions (Figure 1B, 1D and Figure 2C). Structured illumination microscopy showed beautiful super-resolution images of these multiple nuclear inclusions.

Authors confirmed that the exon 1 HTT mutations had no effect on baseline cell viability, though basal respiratory chain deficits were observed using the cell mito stress test assay. Additional investigation into the underlying mechanism of this impairment in neurons was explored by assaying the electron chain components, where results indicated that complex I, complex II+III, and complex III activity was reduced though total CoQ10 status was unaffected. Furthermore, they showed ATP production, maximal respiration rate, non-mitochondrial respiration, and proton leak was negatively impacted and decreased in the 122 CAG line in NSCs, though these differences disappeared post-differentiation. They explored potential links of this mitochondrial dysfunction back to the intranuclear mHTT inclusion body formation phenotype but found

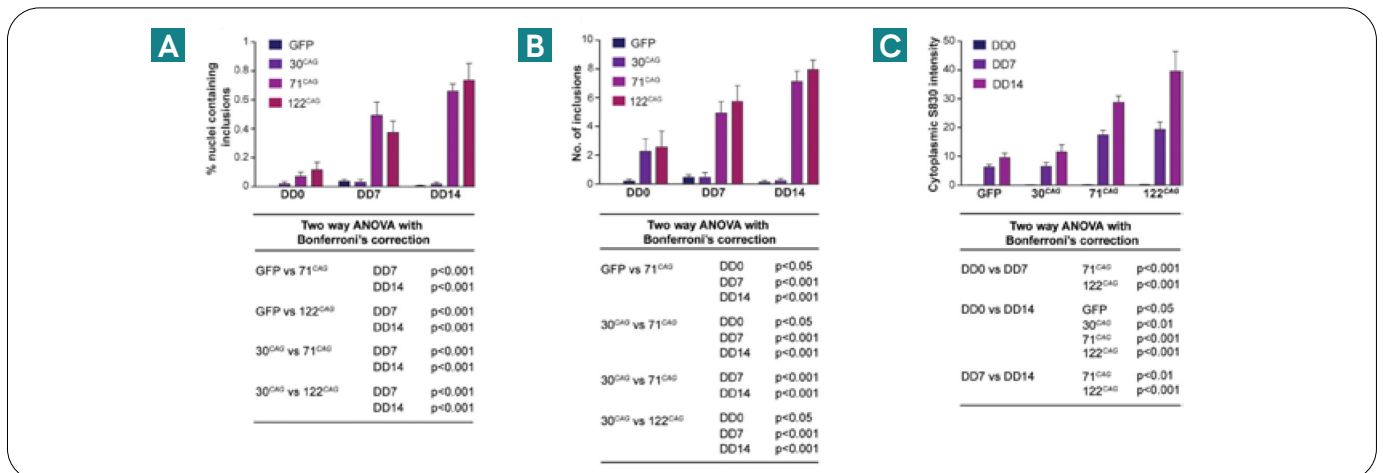


Figure 2: Taken from Figure 3, quantitation of the accumulation of HTT inclusion bodies as % of total nuclei, number of inclusions, and S830 intensity over time (DD0 to DD14) and exon 1 HTT CAG-length dependent manner. Additional method and image details can be found in the method and figure legend of doi.org/10.1096/fj.201902277RR.

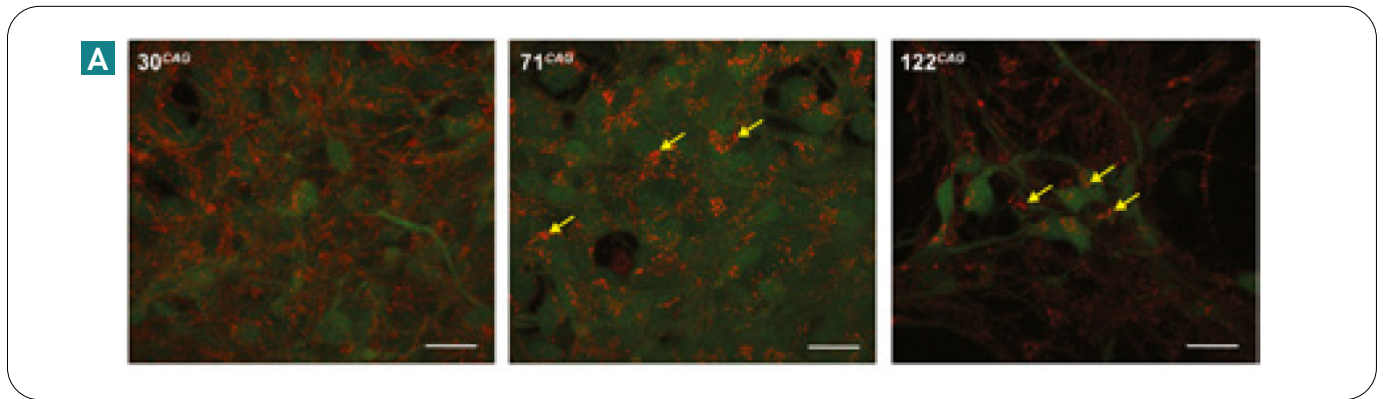


Figure 3: Taken from Figure 7, visual observation of mitochondrial morphology following pre-incubation with TMRM, where mitochondria appeared aggregated and dysmorphic in 122 CAG cells compared to the typical reticular appearance of mitochondria in 30 CAG cells. Additional method and image details can be found in the method and figure legend of doi.org/10.1096/fj.201902277RR.

that increasing endogenous ATP levels via supplementation had no direct effect on IB formation. They did observe, however, through TMRM imaging exploring basal mitochondrial membrane potential and volumetric analysis that the 71 and 122 CAG neurons contained mitochondria that visually looked irregularly distributed, clumped, or enlarged though this was not attributed solely to an imbalance in the fusion-fission process involving GTPase OPA1 as assessed by western blot analysis.

In conclusion, these human NSC lines efficiently differentiate into neurons and stably express 30, 71, and 122 CAGs of exon 1 HTT while displaying pre-pathological changes such as inclusion bodies and mitochondrial dysfunction. They may become a valuable addition to a researcher's toolbox for their amenability for *in vitro* high-throughput applications such as high-content analysis to help tease out relevant cellular mechanisms contributing to the neurotoxicity of pathogenic CAG repeats in HD as well as in the development novel therapeutics for HD.

References

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