Induced Pluripotent Stem Cells in Huntington’s Disease Research: Progress and Opportunity

Adelaide Tousley and Kimberly B. Kegel-Gleason*

Department of Neurology, Massachusetts General Hospital, Charlestown, MA, USA

Abstract. Induced pluripotent stem cells (iPSCs) derived from controls and patients can act as a starting point for in vitro differentiation into human brain cells for discovery of novel targets and treatments for human disease without the same ethical limitations posed by embryonic stem cells. Numerous groups have successfully produced and characterized Huntington’s disease (HD) iPSCs with different CAG repeat lengths, including cells from patients with one or two HD alleles. HD iPSCs and the neural cell types derived from them recapitulate some disease phenotypes found in both human patients and animal models. Although these discoveries are encouraging, the use of iPSCs for cutting edge and reproducible research has been limited due to some of the inherent problems with cell lines and the technological differences in the way laboratories use them. The goal of this review is to summarize the current state of the HD iPSC field, and to highlight some of the issues that need to be addressed to maximize their potential as research tools.

Keywords: Huntingtin, Huntington’s disease, HTT, induced neuron, induced pluripotent stem cell, neural stem cell, neurodegenerative disorder

INTRODUCTION

Since their development in 2006, induced pluripotent stem cells (iPSCs) have generated excitement due to their potential to produce appropriate cell models to facilitate discovery of novel targets and treatments for human disease [1]. iPSCs derived from controls and patients can act as a starting point for in vitro differentiation into human brain cells without the same ethical limitations posed by embryonic stem cells. The first human Huntington’s disease (HD) iPSCs with mutations of 54 CAG and 72 CAG repeats in the huntingtin gene were created from HD patient fibroblasts in 2008 by Park et al. using retroviral induction of four pluripotency factors: cMyc, Klf4, Oct4, and Sox2 [2]. Since then, numerous groups have successfully produced and characterized iPSCs with different CAG repeat lengths, including cells from patients with one or two HD alleles. HD iPSCs and the neural cell types derived from them recapitulate some disease phenotypes found in both human patients and animal models. Although these discoveries are encouraging, the use of iPSCs for cutting edge and reproducible research has been limited due to some of the inherent problems with cell lines and the technological differences in the way laboratories use them. The goal of this review is to summarize the current state of the HD iPSC field, and to highlight some of the issues that need to be addressed to maximize their potential as research tools (Fig. 1).
The current inventory of HD iPSCs

Early efforts at reprogramming adult cells to iPSCs relied upon lentiviral [3] or retroviral [1] delivery of cDNAs encoding pluripotency factors [4]. iPSCs have been successfully created from a variety of somatic cell types including fibroblasts [1, 3], blood cells [5, 6], renal epithelial cells [7], and keratinocytes [8, 9]. Several reviews discuss the history and recent advances in reprogramming methods used to produce human iPSCs [10–13]. At present, the best characterized HD iPSC lines have been produced from patient fibroblasts using lentivirus [14–16] or retrovirus [2, 17–28] to express a combination of pluripotency factors, including: Oct3/4, Klf-4, Sox2, c-Myc, SSEA4, LIN-28, NANOG, and p53 shRNA (to increase efficiency). However, the potential for off-target effects due to random viral insertions motivated scientists to develop novel non-integrating approaches for delivery including Sendai-virus [29], adenovirus [30] and episomal vectors [6, 31], as well as RNA transfection [32], protein [33], and small molecule [34, 35] based methods [4]. More recently, HD researchers have created and begun to characterize cells produced using non-integrating, episomal vectors for induction of pluripotency [36–42].

Numerous iPSC lines exist with CAG repeats in the range of wild-type to that of HD in the Huntington gene (HTT) (from 17 CAGs to 180 CAGs) [2, 14–28, 36–43]. Many of these cell lines were created by the HD iPSC Consortium and are available through the newly established NINDS Human Cell and Data Repository (NHCPR) including 8 unaffected and 18 HD iPSC lines (the catalog for cell lines can be viewed at the following website: https://stemcells.nindsgenetics.org/). The best described HD iPSC line called HD4 was produced by Park et al. (2008) and contains 72 CAGs [2, 17–20, 23–25, 28]. HD4 was used by the Ellerby laboratory to create two cell lines corrected at the HD locus to 21 CAGs by homozygous recombination [17]. HD4 was also used to create an HD allelic series with 21, 72 and 97 CAGs using gene editing employing the CRISPR/Cas9 system along with an antibody screen to confirm the presence of an expanded polyglutamine region in cell lines [44]. These cell lines together are very useful because they offer an isogenic background on which to delineate effects of
the HD mutation. Although isogenic lines are a “gold standard” for a well-controlled iPSC experiment, the inherent variability that has been found among control iPSCs substantiates a need for more isogenic lines from additional HD iPSCs.

iPSCs derived from individuals with juvenile onset HD (>60 CAGs) have been used more frequently for genomic and proteomic studies than iPSCs from individuals with adult onset HD (39–60 CAG) [2, 14–28, 36–43]. However, only about 5% of HD patients have a juvenile onset (prior to age 20) form of the disease, with associated CAG repeat lengths greater than 60 [45–47]. Tables 1 & 2 show summaries of gene expression and protein changes based upon available data sets. The majority of reported work with iPSCs from those with adult onset HD has been with a single cell line derived by the HD iPSC Consortium in 2012 [15, 36]. This line has 60 CAG repeats which is at the high end of the CAG spectrum for adult onset HD [46, 47]. Additional cell lines with repeats ranging from 43–60 CAGs have been described, they are less well characterized [22, 26, 39, 40, 42, 43]. This is likely in part due to the greater ease of detection of mutant huntingtin protein with longer polyglutamine repeats using antibodies that preferentially recognize extended polyglutamine stretches, and the possibility that longer CAG expansions may give more robust changes, as they do in animal models. At present no iPSCs have been produced from patients bearing “intermediate” CAG repeat lengths (27–35 CAGs) or patients with 36–39 CAGs and reduced disease penetrance. Future work with iPSC derived neuronal cells could provide insight into the molecular changes underlying the behavioral changes identified in intermediate repeat length patients [48], and the differences in disease penetrance with low repeat lengths.

Search for phenotypes unveils numerous CAG repeat length dependent and independent properties

Several common phenotypes exist for neural stem cells (NSCs) (also called neural precursor cells (NPCs)) and neuronal cultures differentiated from adult and juvenile HD iPSCs. NSCs from both adult and juvenile-onset patients have decreased metabolic rate (respiratory capacity or ATP/ADP ratio) [15, 17, 19], reduced filamentous actin protein and cell-cell adhesion [15], as well as parallel changes in gene expression compared to NSCs from controls of genes related to nervous system development, cell assembly and organization [15]. HD neuronal cultures derived from both adult and juvenile-onset iPSCs likewise have increased levels of cell death, caspase 3/7 expression in response to BDNF withdrawal, and altered gene expression in pathways related to cell function/signaling and tissue development [15]. One caveat, however, is that differentiated neuronal cultures are by nature composed of a heterogeneous mixture of cell types and the cell death discovered in this study was not demonstrated to be specific to neurons.

Some unique differences were identified when NSCs from adult onset HD iPSCs were compared to those from juvenile onset HD (Tables 1 & 2). Pathway analysis of gene array data showed alterations in axonal guidance in NSCs from juvenile HD (72, 109 and 180 CAGs) that were not present in NSCs from those with adult onset (60 CAGs) [15, 19]. The axon guidance changes were further associated with altered netrin and netrin receptor expression in 72 CAG NSCs [19]. Analysis of gene array data also indicated the presence of alterations in calcium signaling in an HD NSC line with 60 CAGs, but not in ones with 109 or 180 CAGs [15]. These changes in gene expression may have functional relevance in corresponding iPSC induced neuronal cultures: increasing glutamate levels was associated with increased calcium dyshomeostasis and cell death in neuronal cultures with 60 CAGs but not in those with 180 CAGs [15]. As mentioned above, neuronal cultures were heterogeneous and these changes also were not demonstrated to be specific to neurons.

But, altered calcium signaling after glutamate treatment was also observed in low repeat (37 and 51 CAGs) embryonic stem cell (ESC)-derived forebrain neuronal cultures [49]. Finally, Mattis et al. (2015) identified a Nestin-positive cell population sensitive to BDNF-withdrawal induced cell death that was present only in neuronal cultures from juvenile-onset cell lines (109 and 180 CAGs) but not adult onset (60 CAGs) [36].

Phenotypes that may be relevant to disease have been identified in naïve and differentiated HD iPSCs. These include conditions related to altered cell growth [18, 24], adhesion [15, 17], differentiation [18, 36], protein clearance (proteasomal, autophagic) [15, 16, 25, 40], survival [15, 17–19, 36, 38], oxidative stress/antioxidant response [18, 37, 41], metabolism [14, 15, 17, 19], growth factor signaling [15, 17, 20, 28, 36, 41] mitochondrial fragmentation [14], and electrophysiological properties [15].
| Initial cell Type | Differentiated cell type (Reference) | Cell markers | Clone name (CAG Repeat length) | Gene or protein assay type | Identified gene or protein change in HD cells | Reference |
|------------------|-------------------------------------|--------------|-------------------------------|---------------------------|---------------------------------------------|-----------|
| Retroviral iPSCs  | (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | Undifferentiated iPSCs | Not described. | HD(72CAG), H9 ES (control Embryonic Stem Cells) | Increase in: SOD1, GST, PRX1, PRX2, PRX6, BFT; Decrease in: GPX1, CB1, STMN-1 | [Chae et al., 2012] |
| Retroviral iPSCs  | (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | Undifferentiated iPSCs | Not described. | HD(72CAG), H9 ES (control Embryonic Stem Cells) | Protein changes identified by 2-dimensional electrophoresis play a role in regulation of transcription factors: p53, c-Myc, E2F1, YY1 and NF-κB | [Chae et al., 2012] |
| Lentiviral iPSCs  | (OCT4, SOX2, KLF4, c-MYC, LIN28, NANO) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, Pax6, Sox1, Sox2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD21i.5(21CAG), HD60i.3/HD60i.6(60CAG), HD109i.1 (109CAG) | Gene Microarray Pathway analysis following gene microarray indicates increased caspase-related signaling molecules/TGFβ family genes and decreased cadherin family genes | [An et al., 2012] |
| Lentiviral iPSCs  | (OCT4, SOX2, KLF4, c-MYC, LIN28, NANO) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, Pax6, Sox1, Sox2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD21i.5(21CAG), HD60i.3/HD60i.6(60CAG), HD109i.1 (109CAG) | Whole Transcript Expression Profiling/Ingenuity Pathway Tools | [The HD iPSC Consortium, 2012] |
| Lentiviral iPSCs  | (OCT4, SOX2, KLF4, c-MYC, LIN28, NANO) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, Pax6, Sox1, Sox2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD21i.5(21CAG), HD60i.3/HD60i.6(60CAG), HD109i.1 (109CAG) | Whole Transcript Expression Profiling/Ingenuity Pathway Tools | [The HD iPSC Consortium, 2012] |
| Lentiviral iPSCs  | (OCT4, SOX2, KLF4, c-MYC, LIN28, NANO) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, Pax6, Sox1, Sox2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD21i.5(21CAG), HD60i.3/HD60i.6(60CAG), HD109i.1 (109CAG) | Whole Transcript Expression Profiling/Ingenuity Pathway Tools | [The HD iPSC Consortium, 2012] |

Table 1
Gene/Protein pathways changes in HD iPSCs and derived cells
| iPSC Type | Description | Protein Expression | Neural Differentiation | Whole Transcript Expression Profiling/Ingenuity Pathway Tools | Comments |
|-----------|-------------|--------------------|-----------------------|---------------------------------------------------------------|----------|
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, PAX6, SOX1, SOX2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD21i.5(21CAG), HD60i.3/HD60i.4(60CAG), HD109i.1(109CAG), HD180i.5(180CAG) | (180CAG) vs. (60CAG) NSCs differed in: Calcium Signaling; Genetic Disorder | [The HD iPSC Consortium, 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF/LIF dependent neural stem cells derived from neural rosettes induced by STEM-LIF neural induction media | Nestin | Hdc116(21 CAG corrected), HD4(72CAG) | Weighted Gene Coexpression Network Analysis (RNA-seq) | Altered gene ontology categories: ECM organization, regulation of synapse assembly, mammalian phenotype-abnormal survival or perinatal lethality, axonal guidance, targets of transcription factor SMAD3; Upstream regulators of altered genes: TGF-β, β-estradiol, TNF-α | [Ring et al., 2015] |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Neurons differentiated from dissociated FGF/EGF neurospheres induced by FGF/EGF withdrawal and addition of BDNF, cAMP, Shh, Dkk1, and valproic acid [Aubry et al., 2008] | DARPP32, MAP1, MAP2a/b, Mash1, Bcl-1B, BIII-Tubulin | HD33i.8(33CAG), HD28i.2(28CAG), HD21i.5(21CAG), HD60i.3/HD60i.4(60CAG), HD109i.1(109CAG), HD180i.5(180CAG) | Whole Transcript Expression Profiling/Ingenuity Pathway Tools | Cellular growth and proliferation, cellular assembly and organization, cellular function and maintenance; cell-to-cell signaling and interaction, connective tissue development and function, lipid metabolism; tissue development, embryonic development, organ development, connective tissue disorders, genetic disorder, dermatological diseases and conditions, cellular assembly and organization, cellular function and maintenance, cellular movement | [The HD iPSC Consortium, 2012] |
| Episomal iPSCs (OCT4, SOX2, KLF4, c-MYC, NANOG) | “Striatal” neuronal cells differentiated from IPS derived neurospheres (iPSCs->EBs->neuronal rosettes->neurospheres) by FGF withdrawal in B27 media [Chiu et al., 2015] | TUJ1, MAP2, GABA, GAD65, Calbindin, or DARPP-32 | HD-IPS-A1(43CAG), HD-IPS-B4(43CAG), control IPS 1 & 2 | Gene Microarray | Decrease in RAD51/52 (double stranded break repair), ADORA2A (adenosine 2A receptor), PENK (preenkephalin), ARP2P21 (Ca2+ regulated phosphoprotein), GPR88 (G-protein coupled receptor 88), RGS4 (regulator of G-protein signaling 4), GSTT1/GSTT2 (oxidative stress repair), (glutathione-S transferase) Increase in; DRD1/2 (dopamine receptor 1/2), GAD65 (glutamic acid decarboxylase 2), COMT (Catechol-O-methyltransferase) | [Chiu et al., 2015] |
Table 2

| Initial cell type | Cell type differentiation *(Reference)* | Cell markers | Clone name *(CAG Repeat length)* | Identified gene or protein change in HD cells | Reference |
|-------------------|----------------------------------------|--------------|---------------------------------|---------------------------------------------|-----------|
| Retroviral iPSCs  | Undifferentiated iPSCs *(OCT4, SOX2, KLF4, c-MYC)* | Nanog, Sox2, Oct4, SSEA4, TRA-1-60 | HDc116(21CAG), HD4(72CAG), Hfib2-IPS5 *(control)* | Increase in mRNA levels of cadherin family genes: CDK inhibitor 2B, ID2, ID4, PITX2, THBS1 and LEFTY2 | [An et al., 2012] |
| Retroviral iPSCs  | Undifferentiated iPSCs *(OCT4, SOX2, KLF4, c-MYC)* | Nanog, Sox2, Oct4, SSEA4, TRA-1-60 | HDc116(21CAG), HD4(72CAG), Hfib2-IPS5 *(control)* | Decreased cadherin family mRNA levels: protocadherin 11, protocadherin beta 13, protocadherin gamma subfamily A10 and A2 | [An et al., 2012] |
| Episomal iPSCs    | Undifferentiated iPSCs *(Oct4, Sox2, KLF4, LMYC, LIN28 and shRNA to p53)* | Oct-4, SOX2, NANOG, GDF2, REX01 | ND42228, ND42230 (71CAG); ND42223, ND42224 (109CAG); ND41658 (17/18CAG); ND42245 (21CAG) | Decrease in oxidative stress response related proteins: SOD1, GST, Gpx1; increase in anti-oxidant response proteins: Prx1, Prx2, Prx6 | [Chae et al., 2012] |
| Retroviral iPSCs  | Undifferentiated iPSCs/ES Cells *(OCT4, SOX2, KLF4, c-MYC)& H9 Embryonic Stem Cells* | Not described | HD(72CAG), HD(72CAG), 551-8 IPS*(control iPSCs), H9 ES *(control Embryonic Stem Cells)* | Decrease in ERK phosphorylation (Thr202/Tyr204), no change in β-catenin levels or phosphorylation (Ser33/37), increased SOD1 expression | [Chae et al., 2012] |
| Retroviral iPSCs  | Undifferentiated iPSCs/ES Cells *(OCT4, SOX2, KLF4, c-MYC)& H9 Embryonic Stem Cells* | Not described | HD(72CAG), HD(72CAG), 551-8 IPS*(control iPSCs), H9 ES *(control Embryonic Stem Cells)* | Decrease in cytoplasmic localization, increase in nuclear localization of Prx1 | [Chae et al., 2012] |
| Episomal iPSCs    | Undifferentiated iPSCs *(Oct4, Sox2, KLF4, c-MYC)* | Not described | HD70-2(70CAG), HD180-4(180CAG); CC-1, CE-6 *(controls)* | Elevated p53, p-p53, p-ATM protein levels | [Tidball et al., 2014] |
| Episomal iPSCs    | Striatal-like neurons derived from FGF/EGF dependent neurospheres after FGF withdrawal/induction with Shh, Dkk1, BDNF, cAMP, and valproic acid *(OCT4, SOX2, KLF4, L-MYC, LIN28, p53 shRNA)* | TUJ1, DARPP32 | HD60(60CAG); HD109(109CAG), HD180(180CAG), Controls(28CAG, 33W) | Increased GRIN2B (NMDA receptor subunit) mRNA in 109CAG and 180CAG, NOT 60CAG | [Mattis et al., 2015] |
| Retroviral iPSCs  | FGF/LIF dependent neural stem cells derived from neural rosettes induced by STEM-LIF neural induction media *(OCT4, SOX2, KLF4, c-MYC)* | Nestin | HDc116(21CAG corrected), HD4(72CAG) | Decrease in mRNA specific to striatal development: DARPP32, CTP2, FOXP1, ISL1, TBR1, PAX6, increase in FOX2 mRNA | [Ring et al., 2015] |
| iPSCs Type                  | Neural Stem Cells Description                                                                 | Neural marker | HDiPSCs                                                                 | Decreased mRNA/Protein                                                                 | Reference          |
|-----------------------------|-----------------------------------------------------------------------------------------------|---------------|-------------------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------|
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF and LIF dependent NSC produced from selection of neuronal rosettes apparent after plating of EBs (serum dep.)  
(Zhang et al., 2010) | Nestin | HDc116(21CAG), HD4(72CAG), HFib2-IPS5 (control)                       | Decreased TGFβ1 mRNA                                                                   | An et al., 2012    |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF and LIF dependent NSC produced from selection of neuronal rosettes apparent after plating of EBs (serum dep.)  
(Zhang et al., 2010) | Nestin | HDc116(21CAG), HD4(72CAG), HFib2-IPS5 (control)                      | Decrease in BDNF mRNA                                                                    | An et al., 2012    |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-Myc) | FGF/EGF dependent neural stem cells grown as a monolayer after neuroepithelial induction with SB431542 and Noggin  
(Feyeux et al., 2012) | Not described | HD1-IPS4(72CAG), RC9 and SA-01 (WT Embryonic Stem Cells)               | Decrease in BDNFII and BDNFIV mRNA                                                      | Charbord et al., 2013 |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | LIF dependent neural precursor cells induced by small molecule inhibitors: SB431542 and CHIR99021  
(Li et al., 2011) | Nestin | CAG33(33CAG) and CAG180(180CAG)                                      | Decrease in MAO-A and MAO-B mRNA levels, increase in MAO-A and MAO-B activity          | Ooi et al., 2015    |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-Myc) | FGF/LIF dependent neural stem cells derived from neural rosettes induced by STEM-LIF neural induction media | Nestin | HDc116(21CAG corrected), HD4(72CAG)                                 | Increase in TGF-β precursor, dimer and monomer protein levels; increased SMAD-2 phosphorylation, increased SMAD-2 phosphorylation after human recombinant TGF-β stimulation | Ring et al., 2015   |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF and LIF dependent NSC produced from selection of neuronal rosettes apparent after plating of EBs (serum dep.)  
(Zhang et al., 2010) | Nestin | HDc116(21CAG), HD4(72CAG), HFib2-IPS5 (control)                     | Decreased n-cadherin mRNA, and n-cadherin protein                                        | An et al., 2012    |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres  
(Zhang et al., 2010) | Nestin, PAX6, SOX1, SOX2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD60i.3/HD60i.4(60CAG), HD180i.5/HD180i.7(180CAG) | Decreased actin protein as measured by a phalloidin binding assay | The HD iPSC Consortium, 2012 |

(Continued)
| Initial cell type | Cell type differentiation (Reference) | Cell markers | Clone name (CAG Repeat length) | Identified gene or protein change in HD cells | Reference |
|------------------|---------------------------------------|--------------|-------------------------------|---------------------------------------------|-----------|
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF/LIF dependent neural stem cells derived from neural rosettes induced by STEM-LIF neural induction media | Nestin | HDc116(21CAG corrected), HD4(72CAG) | Increased netrin mRNA, increase in netrin receptor: DCC, UNC5D mRNA, decrease in netrin receptor mRNA: UNC5CL, UNC5C, UNC5B | [Ring et al., 2015] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | Telencephalic progenitors induced by small molecule inhibitors: SB431542, LDN193189, IWR1 | Not described | CSD83iCTRL33n1(33CAG) and CS21HD60n5(60CAG) | Increase in Akt/mTOR signalling effector RTP801(REDD1) protein levels | [Martín-Flores et al., 2015] |
| Episomal iPSCs (OCT4, SOX2, KLF4, c-MYC, NANOG) | “Striatal” neuronal cells differentiated from IPS derived neurospheres (iPSCs->embryoid bodies->neural rosettes->neuropheres) by FGF withdrawal in B27 media | TUJ1, MAP2, GABA, GAD65, Calbindin, or DARPP-32 | HD-IPS-A1(43CAG), HD-IPS-B4(43CAG), control IPS 1 & 2 | Decrease in A2AR mRNA expression, decrease in A2AR protein levels | [Chiu et al., 2015] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | Neuronal cells derived after FGF withdrawal from neurospheres formed by selection of PA6 cell co-culture induced neural rosettes [Kawasaki et al., 2000] | MAP2 | HD(72CAG), HD(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells) | Decrease in western blot detected cytoskeleton associated protein expression: Cfl-1, Stmn-1, Facn-1 and Sept-2, confirmed by immunocytochemistry | [Chae et al., 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | Neuronal cells derived after FGF withdrawal from neurospheres formed by selection of PA6 cell co-culture induced neural rosettes [Kawasaki et al., 2000] | MAP2 | HD(72CAG), HD(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells) | Decrease in oxidative stress response related proteins: SOD1, GST, Gpx1; increase in anti-oxidant response proteins: Prx1, Prx2, Prx6 | [Chae et al., 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | Neuronal cells derived after FGF withdrawal from neurospheres formed by selection of PA6 cell co-culture induced neural rosettes [Kawasaki et al., 2000] | MAP2 | HD(72CAG), HD(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells) | Increase in double strand DNA damage related mRNA: ATM, BFT3; Increase in BFT3 protein and phosphorylation of ATM, p53 and H2AX; Increase in apoptosis related protein cleavage of: Bid, caspase-3, caspase-7 and caspase-9 | [Chae et al., 2012] |
### Table 3

**Functional phenotypes in HD iPSCs and derived cells**

| Initial cell type | Cell type differentiation (*Reference*) | Cell markers | Clone name (CAG repeat length) | HD cell reference | HD cell phenotype | reference |
|-------------------|----------------------------------------|--------------|-------------------------------|------------------|-------------------|-----------|
| Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Undifferentiated iPSCs | NANOG, TRA1-81, OCT4 | HD-iPSChom4F1/2 and 3F1 (42/44CAG), HD-iPShom4F1/2 and 3F1 (42/44CAG), HD-iPShomF3 (39/43CAG), HD-iPSHet3F1 (17/45CAG), WT-iPShom3F1 (15/17CAG), WT-iPShom4F1 (15/18CAG) | No difference number of mitotic cells as measured by PH3 phospho-histone expression (ICC) | [Camnasio et al., 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | Undifferentiated iPSCs | Nanog, Sox2, Oct4, SSEA4, TRA-1-60 | HDc116 (21CAG), HD4 (72CAG), HFib2-iPS5 (control) | No difference in proliferation rate (BRDU assay) | [An et al., 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | Undifferentiated iPSCs | Nanog, Sox2, Oct4, SSEA4, TRA-1-60 | HDc116 (21CAG), HD4 (72CAG), HFib2-iPS5 (control) | Decreased ERK phosphorylation in response to FGF | [Zhang et al., 2010] |
| Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Undifferentiated iPSCs | NANOG, TRA1-81, OCT4 | HD-iPSChom4F1/2 and 3F1 (42/44CAG), HD-iPShom4F1/2 and 3F1 (42/44CAG), HD-iPShomF3 (39/43CAG), HD-iPSHet3F1 (17/45CAG), WT-iPShom3F1 (15/17CAG), WT-iPShom4F1 (15/18CAG) | Increased lysosomal activity as measured by LysoTracker® Red DND-99 fluorescence, increased in response to 48 hrs sucrose treatment | [Camnasio et al., 2012] |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | Undifferentiated iPSCs | NaNOG, TRA1-81, OCT4 | HD-iPSChom4F1/2 and 3F1 (42/44CAG), HD-iPShom4F1/2 and 3F1 (42/44CAG), HD-iPShomF3 (39/43CAG), HD-iPSHet3F1 (17/45CAG), WT-iPShom3F1 (15/17CAG), WT-iPShom4F1 (15/18CAG) | No difference in caspase 3/7 (*** also no unique difference in homozygote vs. heterozygote cells) | [Camnasio et al., 2012] |
| Episomal iPSCs | Undifferentiated iPSCs | Not described. | HD35 (5,7,9) (35CAG), HD57 (1,4,6,7,15) (57CAG), HD58 (1,3,13,19,29,21,31,34) (58CAG), HD70 (2,5,11) (70CAG), HD180 (1,3,4,6,10,14,16) (180CAG), CA (11,24,26,30), CC (1,2,3,5) CD (2,3,10,12), CE (2,3,4,6), C-ESS (6,10) (controls) | Increase chromosomal abnormalities after reprogramming based upon karyotype data, no significant differences in number of chromosomal abnormalities accumulated after continuous passaging | [Tidball et al., 2016] |

(Continued)
| Initial cell type | Cell type differentiation (Reference) | Cell markers | Clone name (CAG repeat length) | HD cell phenotype | Reference |
|------------------|--------------------------------------|--------------|-------------------------------|------------------|-----------|
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | Undifferentiated iPSCs | NANOG, TRA1-81, OCT4 | HD-iPS4GdF1/2 and 3F1 (42/44CAG), HD-iPShom3F3 (39/43CAG), HD-iPShet3F1 (17/45CAG),WT-iPShom3F1 (15/17CAG) | No difference in caspase 3/7 (*** also no unique difference in homozygote vs. heterozygote cells) | [Cannasio et al., 2012] |
| Episomal iPSCs | Undifferentiated iPSCs | Not described. | HD35(5,7,9) (35CAG), HD57(1,4,6,7,15) (57CAG), HD58(1,3,13,19,21,31,34) (58CAG), HD70(2,5,11) (70CAG), HD180(1,3,4,6,10,14,16) (180CAG); CA(11,24,26,30), CC(1,2,3,5) CD(2,3,10,12), CE-(2,3,4,6), C-ESS(6,10) (controls) | Increase chromosomal abnormalities after reprogramming based upon karyotype data, no significant differences in number of chromosomal abnormalities accumulated after continuous passaging | [Tidball et al., 2016] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF and LIF dependent NSC produced from selection of neuronal rosettes apparent after plating of EBs (serum dep.) | Nestin | HDc116(21CAG), HD4(72CAG), HFib2-IPS5 (control) | Decreased maximum respiratory rate as measured by oxygen consumption rate after uncoupling via FCCP treatment | [An et al., 2012] |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, PAX6, SOX1, SOX2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD60i.3/HD60i.4(60CAG), HD180i.5/HD180i.7(180CAG) | Decreased intracellular ATP, decreased ATP/ADP ratio | [The HD iPSC Consortium, 2012] |
| iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | FGF dependent neurospheres (iPSCs->EBs->neuronal rosettes->neurons) | TUJ1,MAP2, GABA, GAD65,Calbindin, or DARPP-32 | HD-IPS-A1 (43CAG), HD-IPS-B4 (43CAG) | No difference in expansion rate | [Chiu et al., 2015] |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres | Nestin, PAX6, SOX1, SOX2, Musashi | HD33i.8(33CAG), HD28i.2(28CAG), HD60i.3/HD60i.4(60CAG), HD180i.5/HD180i.7(180CAG) | Decreased sphere formation from single cell suspension after 12 hours | [The HD iPSC Consortium, 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF/LIF dependent neural stem cells derived from neural rosettes induced by STEM-LIF neural induction media | Nestin | HD116(21CAG corrected), HD4(72CAG) | Increase in TGF-β precursor, dimer and monomer protein levels; increased SMAD-2 phosphorylation, increased SMAD-2 phosphorylation after human recombinant TGF-β stimulation | [Ring et al., 2015] |
Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells

FGF-dependent neurospheres derived from PA6 cell co-culture induced neural rosettes, transplanted into QA-lesioned Sprague-Dawley rats [Kawasaki et al., 2000]

Nestin, DARPP32, GABA, MAP2, GAD65/67, SVP38

HD(72CAG), HD2(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells)

Form EM-48 positive aggregates, 40 weeks after transplantation [Jeon et al., 2012]

Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs

FGF/EGF dependent neurosphere derived from PA6 cell co-culture induced neural rosettes [Kawasaki et al., 2000]

Nestin, DARPP32, GABA, MAP2, GAD65/67, SVP38

HD(72CAG), HD2(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells)

Increased lysosomal activity as measured by LysoTracker® Red DND-99 fluorescence [Camnasio et al., 2012]

Episomal iPSCs

FGF/EGF dependent neural stem cell monolayer derived from dissociated neurospheres

Nestin, PAX6, SOX1, SOX2, Musashi

HD33i.8 (33 CAG), HD60i.3/HD60i.4 (60 CAG), HD180i.5/HD180i.7 (180 CAG)

Increased cleaved caspase-3 [The HD iPSC Consortium, 2012]

Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells

Neuronal cells derived after FGF withdrawal from neurospheres formed by selection of PA6 cell co-culture induced neural rosettes [Kawasaki et al., 2000]

MAP2

HD(72CAG), HD2(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells)

Form less MAP2+ neurons, with shorter neurite length [Chae et al., 2012]

Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG)

Neuronal cells differentiated from dissociated FGF/EGF dependent neurospheres after FGF/EGF withdrawal and addition of ascorbic acid and cAMP

MAP2a/b, GABA

HD33i.8 (33 CAG), HD60i.3/HD60i.4 (60 CAG), HD180i.5/HD180i.7 (180 CAG)

180CAG cells fail to produce inward/outward currents and fire action potentials during whole cell patch clamp recordings [The HD iPSC Consortium, 2012]
## Table 3 (Continued)

| Initial cell type | Cell type differentiation (Reference) | Cell markers | Clone name (CAG repeat length) | HD cell phenotype | Reference |
|-------------------|--------------------------------------|--------------|-------------------------------|-------------------|-----------|
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Neuronal cultures differentiated from dissociated FGF/EGF neurospheres induced by FGF/EGF withdrawal and addition of BDNF, cAMP, Shh, Dkk1, and valproic acid [Aubry et al., 2008] | DARPP32, MAP1, MAP2a/b, Mash1, Bel-1B, BIITubulin | HD33i.8(33CAG), HD180i.5(180CAG) | Increase in cell death as measured by number of condensed nuclei | [The HD iPSC Consortium, 2012] |
| Episomal iPSCs (OCT4, SOX2, KLF4, L-MYC, LIN28, p53 shRNA) | Striatal-like neurons derived from FGF/EGF dependent neurospheres after FGF withdrawal/induction with: Shh, Dkk1, BDNF, cAMP, and valproic acid | TUJ1, DARPP32 | HD60(60CAG), HD109(109CAG), HD180(180CAG), Controls(28CAG, 33W) | Increase in persistent Nestin+cells after 42 days of differentiation; Nestin+cells are more susceptible to BDNF-withdrawal induced increased cell death as measured by number of TUNEL+nuclei | [Mattis et al., 2015] |
| Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Differentiated Neurons, Day 30 of neuronal induction using small molecule SB431542 and Noggin | BIII Tubulin, MAP2, PAX6, Nestin, GABA | HD-iPS<sup>hom</sup>4F1/2 and 3F1 (42/44CAG), HD-iPS<sup>het</sup>3F1 (17/45CAG), WT-iPS<sup>hom</sup>3F1 (15/17CAG) | Increased macroautophagy as measured by increased LC3 BII/LC3 B I protein ratio | [Camnasio et al., 2012] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | Neuronal cells derived after FGF withdrawal from neurospheres formed by selection of PA6 cell co-culture induced neural rosettes [Kawasaki et al., 2006] | MAP2 | HD(72CAG), HD2(72CAG), 551-8 IPS (control iPSCs), H9 ES (control Embryonic Stem Cells) | Increase in cell death as measured by number of TUNEL+nuclei | [Chae et al., 2012] |
| iPSCs (OCT4, SOX2, KLF4, c-MYC, NANOG) | “Striatal” neuronal cells differentiated from IPS derived neurospheres (iPSCs->EBs->neuronal rosettes->neurospheres) by FGF withdrawal in B27 media [Chiu et al., 2015] | TU1, MAP2, GABA, GAD65, Calbindin, or DARPP-32 | HD-iPS A1 (43CAG), HD-iPS B4 (43CAG), control IPS 1 & 2 | No difference in caspase 3 cleavage or H2AX phosphorylation levels | [Chiu et al. 2015] |
| Study | iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Neurons differentiated from dissociated FGF/EGF neurospheres induced by FGF/EGF withdrawal and addition of BDNF, cAMP, Shh, Dkk1, and valproic acid | DARPP32, MAP2a/b, Mash1, Bcl-1B, BIII-Tubulin | HD33i.8(33CAG), HD60i.3/HD60i.4(60CAG), HD180i.5/HD180i.7(180CAG) | Increased risk of cell death as determined by Kaplan-Meier analysis (reversed by addition of 4x concentration of BDNF) | [The HD iPSC Consortium, 2012] |
|-------|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------- |
| Study | Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Neurons differentiated after neuroepithelial induction with small molecules: SB431542 and Noggin, followed by striatal specification with Shh, Dkk, BDNF, ascorbic acid and cAMP [HD iPSC Consortium, 2012] | BIII Tubulin (TUJ1), MAP2, GAD67, DARPP32 | GM05539 (Juvenile onset- no CAG repeat provided), Control (Control iPSCs) | Increased mitochondrial fragmentation in neurites of GAD67+ neurons, shorter neurite length | [Guo et al., 2013] |
| Study | Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Neurons differentiated after neuroepithelial induction with small molecules: SB431542 and Noggin, followed by striatal specification with Shh, Dkk, BDNF, ascorbic acid and cAMP [HD iPSC Consortium, 2012] | BIII Tubulin (TUJ1), MAP2, GAD67, DARPP32 | GM05539 (Juvenile onset- no CAG repeat provided), Control1 (Control iPSCs) | Decreased neurite length in DARPP32+ neurons | [Guo et al., 2013] |
| Study | Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Neurons differentiated after neuroepithelial induction with small molecules: SB431542 and Noggin, followed by striatal specification with Shh, Dkk, BDNF, ascorbic acid and cAMP [HD iPSC Consortium, 2012] | BIII Tubulin (TUJ1), MAP2, GAD67, DARPP32 | GM05539 (Juvenile onset- no CAG repeat provided), Control1 (Control iPSCs) | Decreased mitochondrial membrane potential (MMP), increased mitochondrial ROS production, decreased ATP, increased cell death (lactate dehydrogenase level) | [Guo et al., 2013] |
| Initial cell type | Cell type differentiation (Reference) | Cell markers | Clone name (CAG repeat length) | Stressor | HD cell phenotype | Reference |
|------------------|--------------------------------------|--------------|-------------------------------|----------|------------------|-----------|
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | Undifferentiated iPSCs | Nanog, Sox2, Oct4, SSEA4, TRA-1-60 | Hdc116(21CAG), HD4(72CAG), HFib2-IPS5 (control) | Growth factor withdrawal | No difference in caspase3/7 activation following serum/growth factor withdrawal | An et al., 2012 |
| Episomal iPSCs | Undifferentiated iPSCs | Not described | HDS8-19(58CAG), HD70-2(70CAG), HD180-4(180CAG); CC-1, CD-2, CE-6 (controls) | Induced DNA breakage | Significantly less of a loss of cell viability in response to neocarzinostatin exposure at multiple concentrations, compared to control cell lines | Tidball et al., 2016 |
| Episomal iPSCs | Undifferentiated iPSCs | Not described | HD70-2(70CAG), HD180-4(180CAG); CC-1, CE-6 (controls) | Induced DNA breakage | Significantly increased p-p53 and total p53 protein, and p-ATM after neocarzinostatin treatment at multiple concentrations, similar magnitude of change after neocarzinostatin induction as control cells, but HD cells had higher baseline p-p53, p53 and ATM expression | Tidball et al., 2016 |
| Episomal iPSCs | Astrocytes | GFAP, s100β | F-HD-IPS (50CAG), D-HD-IPS (109CAG) | Inhibition of Protein Clearance | Increase in autophagy; increase in LC3+ cytoplasmic vacuoles after chloroquine treatment | Juopperi et al., 2012 |
| Episomal iPSCs | Striatal-like neural progenitors induced by small molecules SB31542 and purmorphamine [Delli Carri et al., 2013] | ISLET1, PAX6, FOXG1 | HD70-1(70CAG), HD180-4(180CAG) | Altered Ion Concentration | Decreased p-p53(Ser15), p-CHK2(Thr68) and p-Akt (Ser473) after treatment with Mn²⁺; Decreased Mn²⁺ uptake after 24 hrs; No effect upon caspase3 activation or PARP phosphorylation | Tidball et al., 2014 |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF and LIF dependent NSC produced from selection of neuronal rosettes apparent after plating of EBs (serum dep.) [Zhang et al., 2010] | Nestin | Hdc116(21CAG), HD4(72CAG), HFib2-IPS5 (control) | Growth factor withdrawal | Increased caspase3/7 activity and decreased maximal respiratory capacity after FGF/LIF withdrawal | An et al., 2012 |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF/LIF dependent neural stem cells derived from neural rosettes induced by STEM-LIF neural induction media | Nestin | Hdc116(21CAG corrected), HD4(72CAG) | Growth factor withdrawal | Increase in cell death as indicated by increase in TUNEL+cells and elevated caspase 3/7 activation in response to FGF/LIF withdrawal | Ring et al, 2015 |
| iPSC Method | Neurosphere Characterization | Neuronal Differentiation | Growth Factor Withdrawal | Other Findings |
|-------------|------------------------------|--------------------------|--------------------------|---------------|
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) & H9 Embryonic Stem Cells | FGF dependent NSC produced from selection of neuronal rosettes after plating of EBs (serum dep.) | Nestin, SOX1, PAX6 | HD IPS (72CAG), WT IPS, H9 Embryonic Stem Cells | Elevated caspase 3/7 activity in response to EGF withdrawal |
| Episomal iPSCs | Striatal-like neural progenitors induced by small molecules SB31542 and purmorphamine [Delli Carri et al., 2013] | ISLET1, PAX6, FOXG1 | HD70-1 (70CAG), HD180-4 (180CAG) | Elevated p-p53 (Ser15), p-CHK2 (Thr68) and p-H2AX (Ser139) in response to double stranded breaks after neocarzinostatin treatment |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Striatal-like neural progenitors induced by small molecules SB31542 and purmorphamine | DARPP32, MAP1, MAP2a/b, Mash1, Bcl-1B, IIIIs-Tubulin | HD33i.8 (33CAG), HD180i.5 (180CAG) | Increased in cell death as measured by number of condensed nuclei in response to autophagy inhibition (3-MA) but not proteasome inhibition (lactacystin) |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Neuronal cells differentiated from dissociated FGF/EGF dependent neuropheres after FGF/EGF withdrawal and addition of ascorbic acid and cAMP | MAP2a/b, GABA | HD33i.8 (33CAG), HD60 (60CAG), HD180i.5 (180CAG) | Increased calcium dyshomeostasis, further increased in 60CAG in response to 14 days glutamate pretreatment; increased cell death following glutamate pulses as measured by increased in TUNEL+nuclei |
| Episomal iPSCs (OCT4, SOX2, KLF4, LIN28, p53 shRNA) | Striatal-like neurons derived from FGF/EGF dependent neuropheres after FGF withdrawal/induction with: Shh, Dkk1, BDNF, cAMP, and valproic acid | TUJ1, DARPP32 | HD60 (60CAG), HD109 (109CAG), HD180 (180CAG), Controls (28CAG, 33W) | BDNF withdrawal induces increased cell death as measured by number of TUNEL+nuclei |

(Continued)
| Initial cell type                  | Cell type differentiation (Reference)                                                                 | Cell markers                                                                 | Clone name (CAG repeat length)                           | Stressor                          | HD cell phenotype                | Reference                                      |
|-----------------------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------------------------------------------------|-----------------------------------|----------------------------------|-----------------------------------------------|
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Neurons differentiated from dissociated FGF/EGF neurospheres induced by FGF/EGF withdrawal and addition of BDNF, CAMP, Shh, Dkk1, and valproic acid [Aubry et al., 2008] | DARPP32, MAP1, MAP2a/b, Mash1, Bcl-1B, BIII-Tubulin | HD33i.8(33CAG), HD109i.1(109CAG), HD180i.5(180CAG) | Growth factor withdrawal | Increased cell death after 48 hrs. BDNF withdrawal as measured by condensed nuclei | [The HD iPSC Consortium, 2012] |
| Lentiviral iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, NANOG) | Neurons differentiated from dissociated FGF/EGF neurospheres induced by FGF/EGF withdrawal and addition of BDNF, cAMP, Shh, Dkk1, and valproic acid [Aubry et al., 2008] | DARPP32, MAP1, MAP2a/b, Mash1, Bcl-1B, BIII-Tubulin | HD33i.8(33CAG), HD28i.2(28CAG), HD60i.3/HD60i.4(60CAG), HD180i.5/HD180i.7(180CAG) | Growth factor withdrawal | Increased caspase-3/7 activity after 24 hrs BDNF withdrawal | [The HD iPSC Consortium, 2012] |
| Episomal iPSCs                    | Mixed neuronal cells derived from neurospheres [The HD iPSC Consortium, 2012]                             | GFAP, TUJ1, DARPP32                                                         | GM09197 (180/18CAG), ND39258 (109/9CAG), GM05400 (218CAG) | Growth factor withdrawal | Increased cell death as measured by number of TUNEL+ nuclei after BDNF withdrawal | [Lu et al., 2014]                                |
| iPSCs (OCT4, SOX2, KLF4, c-MYC, NANOG) | “Striatal” neuronal cells differentiated from IPS derived neurospheres (iPSCs->Embryoid bodies->neuronal rosettes->neurospheres) by FGF withdrawal in B27 media [Chiu et al., 2015] | TUJ1, MAP2, GABA, GAD65, Calbindin, or DARPP-32 | HD-IPS-A1 (43CAG), HD-IPS-B4 (43CAG), control IPS 1 & 2 | H202 Treatment | Dose dependent increase in γH2AX levels after H202 treatment | [Chiu et al., 2015] |
Conclusions about HD iPSC phenotypes have been drawn from a maximum of three HD cell lines compared to one or two control lines in a given publication [15, 16, 19, 36, 41, 42], with many groups using a single cell line or pair of cell lines compared to controls [14, 17, 18, 20–22, 24, 24–28, 37–40, 43] (see also Tables 1–4 for number of cell lines used in specific studies with their corresponding references). A major problem therefore in phenotype characterization has been the limited efforts to validate results in multiple iPSCs with similar CAG repeats or a range of CAG repeat lengths.

In future studies, one solution to this problem is to use multiple control and HD cell lines. The number of cell lines used should be determined using a power analysis based on data from individual phenotypes, just as in animal studies. Robust phenotypes may require testing far fewer iPSC lines compared to that necessary for more subtle phenotypes. We recommend comparing at least three control and three HD lines in each study. This is established from different individuals as an absolute minimum even for robust phenotypes. Subtle phenotypes may require as many as 10–12 cell lines per group and may require HD lines to have very similar CAG repeats. Ideally at least one genetically corrected iPSC paired with its isogenic HD iPSC should also be included, but two corrected clones are preferred to control for off target effects of the reprogramming strategy could be used (discussed later in “Maximizing the potential for research with HD iPSCs”).

It would also be helpful if researchers currently growing other iPSCs could test published phenotypes in their cells and report conformational studies. Although these types of studies may not be novel and thus may seem of low importance, reproducible HD phenotypes in human cells validated across laboratories is of high value. Several of the current phenotypes have also been shown to occur in animal HD models, increasing confidence that they are real changes that may be important for HD; however, even for changes already shown in other HD models, demonstrating these changes in just one human HD iPSC line is of limited use.

**Varied methods create mixed results: comparing apples to apples or apples to oranges?**

Considerable variability exists in the culture methods used to reprogram somatic cells, and to derive NSCs and neurons from HD iPSCs. This limits the ability to draw broad conclusions from the existing published data. Though reprogramming techniques have been consistent: 17 of 24 publications HD cell lines were produced by retroviral or lentiviral reprogramming, the terms ‘NSC/NPC’ and ‘neuron’ were used to describe cell populations that varied in a multitude of ways including: protein markers of differentiation, growth factor dependence, and surface matrix [50–54]. The marked differences that exist in culture protocols can be explained to some extent by the ongoing progress made in the iPSC field to improve differentiation efficiency and specificity. Nonetheless, as with iPSCs from patients with other neurodegenerative diseases, the relative homogeneity of cell lines (i.e. few patient donors or few cell lines used during comparisons), when combined with variability in reprogramming and differentiation methods, may disguise subtle but important phenotypes, and make it challenging to determine which phenotypes are disease-relevant rather than due to culture conditions, clonal variation, or genetic background of the patient [55].

There are several protocols for differentiation of iPSCs favoring certain neuronal subtypes including medium spiny neurons (MSNs), the GABA-ergic projection neurons of the striatum that is particularly vulnerable in HD [15, 24, 36, 43, 56–64]. Table 5 summarizes salient features of MSN differentiation protocols. The source of MSNs in the developing human striatum is the lateral geniculate eminence (LGE) of the ventral telencephalon [65, 66]. LGE precursors are characterized by expression of several transcription factors including: GSX2, DLX2, ISL1, BCL11B, BCL11B(CTIP2), FOXP1, and FOXP2 [67]. Mature MSNs are characterized by the co-expression of several protein markers including: DARPP32, BCL11B, FOXP1 or FOXP2 [68, 69].

Driving the differentiation of MSNs from human pluripotent stem cells requires the manipulation of several signaling pathways, including Wnt/β-catenin, and Sonic Hedgehog (Shh), and bone morphogenic protein (BMP) well described in a recent review by Fjodorova et al. (2015) [70]. The inconsistent and frequently limited efficiency of existing MSN differentiation protocols, ranging from 5–80% MSN yield, see Table 5) is in part due to the complexity of factors that combine to specify MSN fate. Differentiation of MSNs from iPSC is a lengthy process, with protocols ranging from 3–16 weeks. Production of MSNs from iPSCs requires the derivation of LGE-like neural precursor cells, rather than those approximating the medial or caudal geniculate eminence, the maturation of the neurons rather than maintenance of
| Initial cell Type | Method | Differentiation length | Differentiation efficiency | Transplantation study | Reference |
|------------------|--------|------------------------|---------------------------|-----------------------|-----------|
| Control human ESC | Stromal cell co-culture to produce neural precursor cells, then treated by BDNF, Shh and DKK1, and allowed to mature in the presence of BDNF but without Shh and DKK1 | 62 days | 22% MAP2+ of which 53% also DARPP32+ | Cells were transplanted at various points (23–72 days) of differentiation into quinolinic (QA)-lesioned rats to determine optimal differentiation stage (e.g. neuroepithelial to mature neuron). Tissue was likewise analyzed at multiple timepoints post-graft. Cells transplanted from early differentiation stages produced “tumor-like regions” in host brains, not present in later stages. Grafts of differentiation Day 45 cells produced DARPP32+ neuron-like cells which co-expressed MAP2 and NeuN. Furthermore, 2 months post-graft animals suffered from weight loss, decreased activity and hemiparesis found to coincide with grafts that expanded outside of the striatum largely due to the proliferation of Nestin+ neural precursor cells | [Aubry et al., 2008] Used with HD iPSC by: [Zhang et al., 2011]; [HD iPSC Consortium, 2013] |
| Control human ESC | Neuroepithelial cells grown as spheres were induced by treatment with media containing N2 supplement, then supplemented with DKK1/Shh to induce ventral telencephalic precursor fate, and terminal differentiation produced in Neurobasal media, containing N2/B27 supplements, along with trophic factors: BDNF, GDNF and IGF1 | 30 days | 40% ISLET1+ cells, with a population of cells co-expressing GAD65/67, MAP2 cells and BIII-Tubulin, and a further sub-population observed to be DARPP32+ | Not reported | [Li et al., 2009] |
| Control immortalized human striatal NSCs (FGF/EGF dependent) | Neuronal differentiation was induced in neural stem cells by growth factor withdrawal (FGF/EGF) in B27-supplemented media. Purmorphamine (a Shh agonist) was added for either 7 or 21 days of differentiation (with longer time period yielding higher percentages of DARPP32+ cells) | 3 weeks | ~30% MAP2+ cells of which 20–25% co-expressed DARPP32 | Not reported | [El-Akabawy et al., 2011] |
| Control human ESC | **Pax-6**+ neuroepithelial cells grown as spheres were induced by treatment with media containing N2 supplement, then disrupted and treated with Shh* or small molecule agonist purmorphamine) to induce LGE-like progenitors, which were then directed towards a neuronal fate using culture conditions containing BDNF, GDNF, cAMP, IGF and valproic acid | 47 days | 93.2% of cells were BIII-Tubulin+, of which 90.2% were GABA+, while 89.7% of GABA+ neurons were also DARPP32+ | Transplanted LGE-like progenitors into striatum of QA-lesioned immunodeficient (SCID) mice, tissue analyzed 4 months post-graft showed the development of human GABA+ neurons in both forebrain and spinal tissue. An estimated 58.6% of cells of grafted cells were GABA+ forebrain cells expressing DARPP32+ co-stained with CTIP+ and Meis2+, while remaining cells were largely Nestin+/Pax6- progenitors along with a small population of neuronal cells positive for calretinin, calbindin or parvalbumin. GABA+ cells formed connections with host neurons (dopaminergic and glutamatergic). Grafted forebrain LGE-like progenitors also reversed motor deficits in QA-lesioned mice. | [Ma et al., 2012] Used with HD iPSC by: [Yao et al., 2015] |
| Human iPSC and ESC: HD(72CAG), HD2(72CAG), 551-8 IPS (control iPSC), H9 ES (control ESC) | FGF-dependent neurospheres were derived from iPSC using PA6 (stromal) cell co-culture induced neural rosettes, with neuronal maturation encouraged by FGF withdrawal and addition of BDNF (Kawasaki et al., 2000) | Not reported | 27% DARPP32+, and co-expressing: GSH-2 or DLX2 | Neural precursor cells were transplanted into striatum of QA-lesioned Sprague Dawley rats, tissue analyzed 12 weeks post-graft. Both HD and WT transplanted cells formed MAP2+ cells, of which some co-expressed DARPP32 and GAD65/67. Both HD and WT cells likewise led to improvements in several behavioral measures 4–12 weeks post graft. Animals receiving HD iPSC did form EM-48 positive aggregates, 40 weeks after transplantation in a later experiment | [Jeon et al., 2012] |
| Human iPSC: HD33i.8(33CAG), HD28i.2(28CAG), HD60i.3/HD60i.4(60CAG), HD180i.5/HD180i.7(180CAG) | Based upon Aubry et al. (2008) protocol, neurons differentiated from dissociated FGF/EGF neurospheres after FGF/EGF withdrawal and addition of BDNF, cAMP, Shh, Dkk1, and valproic acid | 72 days | <10% BIII-Tubulin+ or MAP2a/b+, <5% were DARPP-32/Bcl11B positive in all cell lines, but significant variability was observed between differentiations | Not reported | [HD iPSC Consortium, 2012] Used with HD iPSC by: [Guo et al., 2013] |

(Continued)
| Initial cell type | Method                                                                 | Differentiation length | Differentiation efficiency | Transplantation study                                                                 | Reference                  |
|------------------|------------------------------------------------------------------------|------------------------|----------------------------|---------------------------------------------------------------------------------------|---------------------------|
| Control human ESC and iPSC | Dual SMAD inhibition using SB431542/Noggin (or dorsomorphin/LDN-193189) per Chambers et al. (2009), with ventral telencephalic fate specified by Shh treatment | 60 days                | 51% MAP2ab+, of which 20% are also DARPP32+/CTIP2 (BC111B)+ | Transplanted human ESC derived cells after 38 days of differentiation into striatum of QA-lesioned rats, tissue analyzed at 3, 6 and 9 weeks post-graft. After 3 weeks, some cells maintained proliferative potential (Ki67+), while after 6 weeks the graft contained a large population of MAP2ab+/III-Tubulin+neurons, and after 9 weeks cells expressing telencephalic markers: FOXP1/FOXP2, and MSN marker: DARPP32 were observed. Grafts also improved a behavioral phenotype in lesioned rats: apomorphine-induced turns were decrease in animals receiving grafts | [Delli Carri et al., 2013] Used with HD iPSC by: [Tidball et al., 2014] |
| Control human ESC and iPSC | Dual SMAD inhibition using SB431542/LDN-193189 based upon Chambers et al. (2009), with ventral telencephalic fate specified via Shh and DKK1 treatment | 45 days                | ~40% MAP2+, of which <5% also DARPP32+ | Transplanted human ESC derived cells after 25 days of differentiation into striatum of quinolinic acid lesioned adult nude rats, tissue analyzed 5 months post-graft, showed significant DARPP32+ cells, co-stained with CTIP2 and FOXP1 | [Nicoleau et al., 2013] |
| Control human fibroblasts (post-natal and adult) | Neural induction of human fibroblasts directed by lentiviral infection with miRNAs: Mir-9/9* and miR-124 as described by Yoo et al. (2011), and transcription factors: CTIP2(BCL11B), DLX1, DLX2, and MYT1L (CDM) to further specify DARPP32+ neuronal cell fate | 35 days                | 90% MAP2+ neurons, of which 80% were GABA+, while 70% were DARPP32+ | Transplanted EGFP expressing human fibroblasts into striatum of immunodeficient mice, two weeks after neural induction via lentiviral infection, tissue analyzed Day 50 post-graft, 91% of MAP2+ cells co-expressed DARPP32. Cells were observed to have formed a high density of dendritic spines, to be electrophysiologically active, and developed axonal projections to the substantia nigra and globus pallidus. However, a small number of cells were observed to have migrated outside of the striatal boundary, particularity those failing to express striatal specific (CTIP2) after lentiviral infection | [Victor et al., 2014] |
| Control human iPSC | Two separate differentiation protocols were used to drive a neuronal fate from human iPSC derived neural precursor cells in N2/B27 supplemented media (two step): induction by treatment with valproic acid, followed by maturation in media containing BDNF, GDNF, IGF1 and dibutyryl-cAMP (three step): induction using BDNF, Shh, DKK-1 and rock inhibitor Y-27632, maintenance in BDNF, Shh and DKK-1 containing media, and maturation in media containing only BDNF (two step): 21 days (three step): 60 days | (two step): 51% of MAP2+ cells co-expressed DARPP32 | Not reported | [Lin et al., 2015] |
| Human iPSC: HD-IPS-B4 (43CAG) | Neuroepithelial cells were grown from iPSC after formation of embryoid bodies, and selection for neural rosettes in N2/B27 supplemented media. Neuronal fate was further encouraged by growth factor withdrawal (FGF) 16 weeks | 60–80% DARPP32+/GABA+cells | Not reported | [Chiu et al., 2015] |
| Control human | Dual SMAD inhibition using SB431542/dorsomorphin/LDN with ventral telencephalic fate specified by Activin A treatment 36 days | 20–50% DARPP32+, which coexpress: BCl11B(CTIP2)+,GABA+, GAD65/67+ and PSD95+ Transplanted LGE-like neural precursor cells into the striatum of a rat model of HD, and tissue analyzed 4, 8, and 16 weeks post-graft. DARPP32+ cells were apparent after 8 weeks, and comprised of 49% of graft cells present at 16 weeks. Nestin+cells were observed at 4 and 8 weeks, but had disappeared by 16 weeks. Graft cells received mid-brain dopaminergic and cortical glutamatergic inputs. No behavioral improvement was observed in grafted HD rats | | [Arber et al., 2015] |
| Human iPSC: HD109(109CAG), HD180(180CAG), Controls(28CAG, 33W) | After FGF/EGF dependent NSCs grown as spheres were derived from human iPSC, neuronal differentiation was induced by FGF withdrawal and culture with: Shh, Dkk1, BDNF, cAMP and valproic acid 42 days | DARPP32+/TUJ1+ neuronal cells described, but efficiency not reported | Not reported | [Mattis et al., 2015] |
multipotent neural precursor cells or development of glia, and finally the designation of MSNs rather than the olfactory bulb interneurons [65].

The general pattern used in the currently available MSN differentiation protocols includes neural induction using stromal cell co-culture [24, 56], exposure to growth factors to induce embryoid body/neural rosette formation [15, 36, 43, 58, 60, 71], or dual-SMAD/BMP inhibition [62]. Neural induction is followed by specification of LGE precursor/MSN fate using a combination of factors including Shh and DKK1 (a WNT-inhibitor) [15, 36, 56–58, 71, 72], or more recently Activin A, a TGFβ signaling protein [62]. Alternatively, a recent protocol describes the efficient direct induction of adult human fibroblasts to DARPP32+ neurons using lentiviral infection with miRNAs: MiR-9/9* and miR-124 as described by Yoo et al. [73] to encourage neuronal differentiation, and expression transcription factors: CTIP2(BCL11B), DLX1, DLX2, and MYT1L (CDM) to specifically encourage MSN fate [61] (Table 5).

MSN differentiation protocols have largely been described in control cell lines [57–64], with only a few attempted using HD iPSC lines [14, 15, 27, 28, 37]. Further study is necessary to understand how individual cell lines, HD or control, will respond to each differentiation protocol.

**Drug discovery and the use of stress induced phenotypes**

Drug screens using animal cell models of HD that express endogenous mutant huntingtin (knock-in) or overexpress mutant huntingtin exogenously, as well as those using human cells overexpressing huntingtin, have failed to identify a compound that prevents, slows or reverses disease onset. A well-characterized human neuronal cell model with huntingtin expression from the endogenous gene locus could be a valuable asset in developing a therapeutic. Numerous screens have already been performed using HD iPSC derived cells [14, 19, 20, 25–27, 36–39, 43]. However for each study, testing is often limited to one or two HD iPSC lines, often without a control or corrected cell line for comparison.

Most phenotypic readouts used to test the effectiveness of novel therapies in HD iPSC derived neuronal cells include a stressor such as growth factor withdrawal, oxidative stress, DNA damage, or glutamate toxicity [14, 19, 20, 25–27, 36–39, 43] (Table 6). The effect of a drug on cell response to cytokine treatment [26], induced DNA breakage [37], proteasome inhibition [25], H₂O₂ or Mn²⁺ treatment [37, 43] and growth factor withdrawal [27, 36, 38, 39], have been investigated. The TNFα inhibitor XPro-1595 lowered cytokine (TNFα and ILβ) induced apoptosis activation and iNOS levels in astrocytes and neurons, respectively, differentiated from iPSCs with 43 CAGs [26]. Adenosine receptor 2A agonists CGS-21680 and APEC produced a dose dependent reduction of oxidative stress toxicity induced by exposure to H₂O₂ in 43 CAG neuronal cultures, as measured by decreased γH2AX induction and caspase3 cleavage [43]. The microRNA 196a (miR196a) was identified as a candidate that might impact HD using microarray data from transgenic HD monkey tissue; miR196a was found to decrease MG-132 induced EM-48 positive huntingtin aggregates in neuronal cultures differentiated from HD iPSCs with 72 CAGs through an unknown mechanism [25]. An ATM (ataxia-telangiectasia mutated) protein inhibitor KU55933 reversed both necrostatin (a DNA damaging agent) induced increases in phosphorylation of p53, CHK2 and γH2AX, and Mn²⁺ decreases in p53 phosphorylation in 70 and 180 CAG “striatal-like” NPCs [37].

Several groups have tested drug effects of growth factor (BDNF or FGF/LIF) withdrawal on cell survival [19, 27, 36, 38, 39]. Although cells were grown in medium containing essential nutrients, acute withdrawal of neurotrophins might also be considered a stress once cells have become dependent upon them. Lu et al. (2014) showed that an alternate ATM protein inhibitor, KU60019, reduced BDNF withdrawal-induced increases in TUNEL-positive nuclei and caspase 3 activation in 109 and 180 CAG mixed neuronal cell cultures [38]. Furthermore, to elucidate mechanisms of BDNF-withdrawal induced cell death in neuronal cultures differentiated from HD iPSCs with 109 and 180 CAG, Mattis et al. (2015) showed reversal of increased TUNEL+nuclei after BDNF withdrawal using several compounds including a calcium chelator (BAPTA), TRKB antibody agonist (αTrkB), MAPK signaling inhibitor
| Initial cell type | Differentiated cell type (Reference) | Cell markers | Clone name (CAG repeat length) | Treatment type | Effect on HD cell phenotype | Reference |
|------------------|--------------------------------------|--------------|-------------------------------|----------------|-----------------------------|-----------|
| Retroviral (OCT4, SOX2, KLF4 and c-MYC) iPSCs | Neuronal cells differentiated in N3 media [Wernig et al., 2002] | BIII-Tubulin | GM23225 (72CAG) | miR-196a delivered by lentiviral (FUW) infection | Decrease in EM-48 positive huntingtin MG-132 induced aggregates, and lowered levels of MAB 2166 detected mutant Htt protein | [Cheng et al., 2013] |
| iPSCs (OCT4, SOX2, KLF4, c-MYC, NANOGL) | “Striatal” neuronal cells differentiated from IPS derived neurospheres (iPSCs → embryoid bodies → neuronal rosettes → neurons) by FGF withdrawal in B27 media [Chiu et al., 2015] | Tuj1,MAP2, GABA, GAD65, Calbindin, DARPP-32 | HD-IPS-A1 (43CAG) | A2AR agonists: CGS-21680 & APEC | Dose dependent reduction in H2O2 induced activation of H2AX, active/pro-caspase3 ratio and TUNEL+ nuclei; effect reversed by A2AR shRNA, A2AR antagonism (SCH5826) or selective PKA inhibition (H-89) | [Chiu et al., 2015] |
| Retroviral (Oct3/4, NANOGL, SOX2, KLF4, c-MYC) iPSCs | Astrocytes derived from iPSCs in B27 media+ ciliary neurotrophic factor [Hsiao et al., 2014] | GFAP | HD IPS- Patient A (43CAG) | TNF-α inhibitor: XPro-1595 | Decrease in cytokine: TNF-α+IL-1β induced iNOS production | [Hsiao et al., 2014] |
| | Neuronal cells derived from NSCs (EBs → NSCs) by FGF withdrawal in B27 media [Hsiao et al., 2014] | BIII-Tubulin | HD IPS- Patient A (43CAG) | TNF-α inhibitor: XPro-1595 | Decrease in cytokine: TNF-α+IL-1β induced caspase3 cleavage | [Hsiao et al., 2014] |
| Retroviral (OCT4, KLF4, SOX2 and c-MYC) iPSCs | Neurons derived from ventral progenitors (induced with Shh and purmorphamino) [Ma et al., 2012] | Tuj1, GABA, DARPP32 | HD IPS (47CAG) HD GPR52 siRNA | Reduces mHtt protein levels (2B7[Yao et al., 2015] and HTRF assay) | Reduces BDNF-withdrawal induced caspase 3 activation, neuronal loss as measured by density of Tuj1/DAPI (+) cells and increases neuronal process complexity (Sholl Analysis) | [Lu et al., 2014] |
| Episomal iPSCs | Mixed neuronal cells derived from neurospheres [The HD iPSC Consortium, 2012] | GFAP, Tuj1, DARPP32 | GM00917 (180/18CAG) ND39258 (109/19CAG) GM05400 (21/18CAG) | ATM inhibitor: KU60019 | Reduces BDNF-withdrawal induced TUNEL(+) nuclei in HD iPSCs derived cells with no effect on control cells | [Lu et al., 2014] |
| Episomal iPSCs | Medium spiny neurons derived using M55-cell co-culture [Aubry et al., 2008] | GFAP, Tuj1, DARPP32 | HD IPS 6084 (60CAG) | PPARδ activator: KD3010 | Reduces BDNF-withdrawal induced cell death as measured by the degree of hoecht-detected nuclear condensation | [Dickey et al., 2015] |
| Initial cell type | Differentiated cell type (Reference) | Cell markers | Clone name (CAG repeat length) | Treatment type | Effect on HD cell phenotype | Reference |
|------------------|--------------------------------------|--------------|--------------------------------|----------------|----------------------------|-----------|
| Episomal iPSCs   | Striatal-like neural progenitors induced by small molecules SB31542 and purmorphamine [Delli Carri et al., 2013] | ISLET1, PAX6, FOXG1 | HD70-1(70CAG), ATM inhibitor: KU55933 HD180-4(180CAG) | Reverses decreased phosphorylation of p53 in response Mn²⁺ concentration increase, and increased phosphorylation of p53, CHK2 and H2AX in response to necarzinostatin treatment | [Tidball et al., 2014] |
| Episomal iPSCs (OCT4, SOX2, KLF4, c-MYC, LIN28, p53 shRNA) | Striatal-like neurons derived from FGF/EGF dependent neurospheres after FGF withdrawal/induction with: Shh, Dkk1, BDNF, cAMP, and valproic acid | TUJ1, DARPP32 | HD109(109CAG), Calcium Chelator: BAPTA HD180(180CAG), TRKB antibody agonist: TrkB MAPK signaling (p38) inhibitor: SB239063 allele specific antisense oligonucleotides: ASO-1, 2 & 3 NMDAR antagonism: memantine; AMPA/Kainate antagonism: CNQX; mGlu I/II glutamate receptor antagonism: S-MCPG | Reverses BDNF withdrawal induced cell death as measured by number of TUNEL+ nuclei | [Mattis et al., 2015] |
| Retroviral iPSCs (OCT4, SOX2, KLF4, c-MYC) | FGF/LIF dependent neural stem cellsNestin derived from neural rosettes induced by STEM-LIF neural induction media | HDc116(21CAG corrected), HD4 (72CAG) | Reverses increase in caspase3/7 activity and decrease in maximal respiratory capacity after FGF/LIF withdrawal | [Ring et al., 2015] |
| Lentiviral (OCT4, SOX2, KLF4, c-MYC) iPSCs | Neurons differentiated after neuroepithelial induction with small molecules: SB431542 and Noggin, followed by striatal specification with Shh, Dkk, BDNF, ascorbic acid and cAMP [HD iPSC Consortium, 2013] | GM05539 (Juvenile onset no CAG repeat provided), Control-1 (Control iPSCs) | Drp1-selective peptide inhibitor: PI 10-TAT | Reverses mitochondrial fragmentation and shortened neurites in GAD67+ neurons, shortened neurites in DARPP32+ neurons, and decreased mitochondrial membrane potential (MMP), increased mitochondrial ROS production, decreased ATP, and increased cell death (lactate dehydrogenase level) in mixed neuronal culture; shRNA to p53 likewise reversed decreases in neurite length in GAD67+ neurons and MMP in mixed neuronal culture, as well as increases in mitochondrial ROS production and cell death | [Guo et al., 2013] |
| Retroviral (OCT4, SOX2, FGF/EGF dependent neural stem cells grown as a monolayer after neuroepithelial induction with SB431542 and Noggin [Feyeux et al., 2012] | Not described | HD1-IPS4 (72CAG), RC9 and SA-01 (WT Embryonic Stem Cells) | Repressor element-1 silencing transcription factor (REST) inhibitor: X5050 | Dose dependent reduction in REST activity (luciferase activity) and increase in RE1 gene mRNA: SNAP25, BDNF, and Syp; similar levels of activity and transcriptional increases to NSC derived from WT Embryonic Stem Cells | [Charbord et al., 2013] |
DNA damage consistent with the neuronal aging [75]. Activated levels of reactive oxygen species (ROS) and iPSC derived dopaminergic neurons resulted in elevated expression of progerin, a mutated form of lamin A associated with premature onset of premature senescence [76]. Furthermore, iPSCs from patients with Hutchinson-Gilford progeria syndrome (HGPS) and atypical Werner syndrome (AWS) which contain mutations in their endogenous LMA gene, have dysmorphic nuclei and premature senescence [76].

**Phenotypes relating to disease mechanism**

It is possible that aging and stress might compound or enhance secondary effects of mutant huntingtin but not affect primary drivers of HD progression. If the underlying primary problem causing disease in humans HD is not related to stress or aging, then screening for compounds that reverse stress induced cell death or age related morphology changes may be insufficient to identify novel therapeutics for HD that target crucial changes. Assessing the ability of a given therapeutic to reverse additional alterations such as cell morphology, cell signaling activity or gene expression in the absence of stress may allow for more confident conclusions about drug efficacy [77]. Additional phenotypes useful for screening could include any of the number of disease relevant phenotypes already identified in HD iPSC derived NSC/NPCs and neurons, though further work is necessary to determine the extent to which these phenotypes can be generalized using HD iPSC lines derived from a larger patient population (Tables 3 & 4).

Utilizing HD iPSCs as a model for early CNS development in HD patients, and determining phenotypes that precede neuronal death may be an equally important and a more readily attainable goal. Study of rodent and human cell models indicate the influence of mutant huntingtin on neural development in general, and specifically related to: premature onset of neural differentiation [78], altered Notch signaling [78] and aberrant mitotic spindle orientation [79, 80]. Recent work has indicated alterations to HD

(SB2390463), NMDAR antagonist (memantine) and AMPA/Kainate antagonist (CNQX), and allele specific antisense-oligonucleotides of mutant huntingtin (ASO1, ASO2, ASO3) [36]. Importantly, the susceptible population in the neuronal culture was Nestin+ radial glia-like cells, not neurons.

Likewise, Yao et al. (2015) explored the potential role of G-protein coupled receptor 52 (GPR52) in mediating mutant huntingtin protein toxicity. They found that siRNA knockdown of GPR52 both reduced mutant huntingtin protein levels, and BDNF withdrawal induced caspase 3 activation in HD iPSC derived neurons with 47 and 70 CAG repeats [27]. In addition, Dickey et al. (2016) described the ability of a PPARγ activator (KD3010) to reduce numbers of condensed nuclei after BDNF withdrawal from 60 CAG derived neuronal cultures [39]. Finally, Ring et al. (2015) assessed the ability of recombinant TGFβ1/TGFβ2 and netrin-1 to reverse increases in caspase3/7 activity and decreases in maximum respiratory capacity that occur after FGF/LIF withdrawal in NSCs with 72 CAGs; TGFβ1/TGFβ2 treatment was likewise found to be beneficial to HD NSCs under unstressed conditions [19].

Aside from the TGFβ experiment by Ring et al. [19], only two other groups have tested the effects of a compound on iPSC phenotypes without the addition of a stressor. Charbord et al. (2013) demonstrated that treatment of 72 CAG NSCs with a Repressor element-1 silencing transcription factor (REST) inhibitor X5050 produced increased RE1 gene expression and reduced REST activity when compared to control ESC derived NSCs [20]. Guo et al. (2013) showed that a Drp1-selective peptide inhibitor, P110-TAT, reversed: neurite shortening, decreases in mitochondrial membrane potential and ATP level, increases in cell death, mitochondrial fragmentation, and mitochondrial reactive oxygen species (ROS) in mixed neuronal cultures derived from iPSCs derived from juvenile HD patient fibroblasts of unreported CAG length (GM05539, Coriell Cell Repository) [14].

In other disease fields, additional phenotypes have been identified using alternative methods of aging iPSC derived neurons well described in a recent review by Studer et al. (2015) [74]. Recently, exogenous expression of progerin, a mutated form of the nuclear envelope protein lamin A associated with disorder of premature aging, has been used to mimic aging [75]. Overexpression of progerin in iPSC derived dopaminergic neurons resulted in elevated levels of reactive oxygen species (ROS) and DNA damage consistent with the neuronal aging [75]. The same experimental procedure was also used in iPSC derived dopaminergic neurons from Parkinson disease (PD) patients bearing PINK1 and Parkin mutations, and expression of progerin unveiled a decrease in dendrite length specific to PD patient neurons, not identified in progerin-negative cells [75]. The introduction of progerin, along with continued work to understand the molecular mechanisms underlying normal aging could lead to an improved understanding of HD neurodegeneration and future cell models for drug screening. Care should be used with interpretation of results identified using progerin overexpression, however, since cells will be burdened with two mutant proteins, one of which is overexpressed. Furthermore, iPSCs from patients with Huntington-Gilford progeria syndrome (HGPS) and atypical Werner syndrome (AWS) which contain mutations in their endogenous LMA gene, have dysmorphic nuclei and premature senescence [76].
patient brains years before symptom onset, including: basal ganglia cell death [81], altered striatal/cortical volume and morphology [81, 82–84], cortico-striatal connectivity [85, 86], decreased cortical inhibition [87], and increased oligodendrocyte number [88, 89]. Early cognitive changes have likewise been identified in HD patients related to altered cortico-striatal circuitry [90]. Behavioral changes and altered synaptic connectivity, preceding the onset of motor symptoms, have also been identified in transgenic mouse models of HD [91–94]. These findings have encouraged the hypothesis that the huntingtin mutation may alter molecular pathways, cell phenotypes and activity during neural development, and the cumulative effects of these changes over time eventually lead to disease onset well described in several reviews: [50, 88, 95, 96].

Genomic and proteomic analyses of HD iPSC and derived neuronal cells support the importance of continued exploration of the role of mutant huntingtin in disrupting the normal differentiation and maturation of human cells. Differentiation of HD iPSCs to NSCs and neurons has uncovered differences in the efficiency [18] and composition of cultures [36], when compared to iPSCs from healthy controls. Furthermore, comparisons of gene microarray and RNAseq data between HD4 (72 CAGs) HD iPSCs and a cell line corrected to 21 CAGs identified axonal guidance [19] and cadherin, TGFβ and SMAD3 signaling [17, 19] as pathways significantly altered in HD cells. Altered expression of genes specifically related to striatal development were also identified in the same cells [19].

Other brain cell types may contribute to HD pathology. Changes to astrocytes, microglia, and oligodendrocytes have been observed in both HD patients [89, 97–101], and transgenic animal models of HD [101–108]. Several protocols have been shown to produce astrocytes [109–114] and oligodendrocytes [115–118] from human iPSCs (45). Two groups have used HD iPSCs to produce GFAP+astrocytes [26, 40] (Tables 3 & 6). Juopperi et al. (2012) reported a CAG-repeat dependent increase in cytoplasmic vacuolization in HD astrocytes derived from 50 CAG and 109 CAG iPSC lines, and related the change to a potential alteration in autophagy as overnight treatment with chloroquine, an autophagy inhibitor, increased LC3+ positive vacuoles in HD cells [40]. Hsiao et al. (2014) showed that a TNF-α inhibitor, XPro-1595, lowers cytokine (TNF-α & IL-β) induced iNOS production in astrocytes derived from iPSCs with 43 CAGs [26]. So far, no work has been published describing HD iPSC derived oligodendrocytes. Further exploration of HD iPSC derived glia, along with co-culture of iPSC derived neurons and glia, may shed light on sources of non-cell autonomous toxicity in HD pathogenesis.

**Technical caveats that may undermine phenotype characterization**

Despite their advantages, unanswered questions about iPSC as a model remain. One area of particular interest for investigating age-related diseases such as HD is obliteration of age from the patient sample. Several studies indicate that induced pluripotency reverses typical age-related phenotypes, such as telomere shortening [119–121] and mitochondrial dysfunction [74, 121, 122]. A total reversal of age related changes such as alterations in levels of oxidative stress, DNA packaging and damage, nuclear morphology and related gene expression changes occurred in fibroblasts differentiated from iPSCs that had been collected from donor patients in different age ranges [74]. Transdifferentiation or directed differentiation to neurons from aged fibroblasts may be one approach to maintaining age [4, 61, 77]. Another issue is the potential for reprogrammed cells to bear an ‘epigenetic memory’ of their somatic cell source [4, 74, 123–125]. A recent study by Kim et al. (2011) indicated that iPSCs retain some residual DNA methylation from their parental cell type (fibroblast or blood cell), rendering them more readily differentiated to their parent cell’s fate [124]. However, recent work also suggests that repeated passages [126] or chromatin modifying agents [127] can diminish or fully reset this ‘epigenetic memory’ [124].

The extent to which the phenomenon of epigenetic memory and limited pluripotency may affect neuronal cells derived from of HD iPSCs is as yet, uncertain. Reprogramming, continuous passage (cell dissociation and re-culturing) to high passage number, and differentiation of iPSCs have been shown to lead to genetic instability [128–132]. Investigators using iPSCs are thus cautioned when using highly passage cells. Encouraging results showed few changes in human iPSCs due to mutant huntingtin. The HD iPSC Consortium (2013) reported an expansion from 110 to 118 CAGs in one HD iPSC derived NSC line, but no changes in iPSC karyotype [15]. Camnasio et al. (2012) reported occasional changes in karyotype in both control and HD iPSCs but stable CAG repeat lengths [16]. This is in contrast to
NSCs established from embryonic brain tissue from homozygous CAG140 knock-in mice which became tetraploid after very few passages in culture [133]. However, a recent finding by Tidball et al. (2016) showed increased somatic instability in HD iPSCs using siRNA targeting p53 to increase the efficiency of reprogramming [42]. Further study is necessary to determine the extent to which additional changes occur in human HD iPSC lines and differentiated cells, but so far the CAG repeat expansion and presence of mutant huntingtin protein do not seem to impose genetic instability.

While progress towards understanding HD mechanisms using iPSC has been unquestionably substantial, there are challenges in drawing broad conclusions about disease pathogenesis from these studies. Limitations on the number of HD and control cell lines used are understandable considering the cost and effort in maintaining human stem cells. However, given the relatively small number of cell lines studied, care should be taken when interpreting results. Although HD is caused by just one gene, the patient donors have diverse genetic backgrounds that could account for many of the changes observed. As with any experiment, sample size increases confidence in results. Efforts should be supported for additional testing of existing cell lines and for continued creation of new cell lines to overcome this limitation. Furthermore, determining the presence of certain phenotypes and susceptibility to specific treatments one day may be possible using iPSC derived neurons allowing for tailored treatments (so called “personalized medicine”) [134]. The availability of cell lines from diverse genetic backgrounds is critical for these efforts.

**Maximizing the potential for research with HD iPSCs**

Genetic correction to create non-disease CAG repeat lengths in iPSCs with the same genetic background as diseased cells in theory removes at least one confounding variable thereby diminishing barriers to identifying less subtle phenotypes. An et al. (2012) reported the successful use of homologous recombination to correct the 72 CAG allele of an HD4 iPSC line to 21 CAG [17]. Recent advances in gene editing technology should make genetic correction of iPSC easier [50–52, 77, 135–138]. The potential for zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly-interspaced short palindromic repeats/Cas 9 (CRISPR/Cas9) to modify human stem cells has been well described [4, 55, 138]. Each method bears limitations in the targeting of guiding DNA/RNA sequences, as well as differing propensities for off-target effects [55, 138], although off target effects are reduced in pluripotent stem cells in contrast to other cells [139]. Current methods of gene editing rely on endogenous machinery to repair DNA breaks produced by an exogenous nuclease (i.e., CRISPR or Fok1) [4, 135, 138]. Several recent findings indicate that the molecular pathways underlying DNA repair may be altered or aberrantly active in HD iPSCs [18, 37, 38, 42, 43], consistent with somatic CAG repeat expansions in post-mortem brain tissue from HD patients, and oxidative DNA damage in HD animal models [140]. These existing changes in HD cells could have adverse effects on the efficiency of genetic corrections, and susceptibility of cells to off-target effects. There are ongoing efforts to target single nucleotide polymorphisms (SNPs) specific to the mutated HD allele and to contract the expanded CAG repeat in heterozygous cell lines [53] in order to create a series of iPSC lines with different CAG expansions lengths on the same genetic background to determine CAG repeat length dependent phenotypes. An HD allelic series 21, 72 and 97 CAGs, was successfully created by An et al., (2014) using the CRISPR/Cas9 gene editing system [44]. One practical problem is that unfortunately many of the existing iPSC lines are not heterozygous for SNPs that could be targeted by CRISPR/Cas9 making allele-specific changes difficult in these lines (our unpublished observations).

Just as an ‘epigenetic memory’ of the somatic cell source may persist in iPSCs after reprogramming, it is as yet unknown if contracting expanded CAG repeats in HD iPSCs will fully reverse the effects of the HD mutation on derived neuronal cells. Using a genetic correction as the control will obscure phenotypes that cannot be reversed since both the mutant and the isogenic corrected line will continue to share the condition; thus they will not be observed as “different”. Analysis of gene and protein expression and epigenetic modifications, comparing both HD iPSC line and iPSC lines derived from healthy patient controls is necessary to understand the extent to which genetic correction in HD iPSC lines can reverse HD phenotypes; it is possible that some phenotypes can be reversed in one cell line with a particular genetic background, but not in another (for in-depth coverage of this topic please see [141]). For HD, only one iPSC (HD4) has been subjected to correction. Correction
by homologous recombination of the iPSC line HD4 from 72 CAGs to 21 CAGs reversed phenotypes of altered TGF-beta and cadherin gene expression [17] and changes to gene ontology categories including synaptic assembly, axonal guidance and SMAD3 signaling [19]. Far fewer changes were found between HD4 and the corrected line, versus HD4 and an unrelated control iPSC line, supporting the notion that an isogenic background is a good control. Studies comparing iPSC derived neurons from healthy controls to Parkinson patients bearing mutations in LRRK2 gene demonstrated that gene expression cluster profiles did not partition with the mutation, indicating the normal genomic variability had a stronger effect than the mutation [142]; meaningful changes were only found using an isogenic corrected control. Additional studies comparing phenotypes of HD4 and the corrected cell line with numerous control cell lines will be informative. These issues are particularly important in considering the potential of genetically corrected HD iPSCs as a tissue source for cell replacement therapy [45, 52, 53, 123].

As an alternative to genetic editing, levels of mutant protein can be lowered using methods that specifically target mutant huntingtin RNA stability and protein expression including: anti-sense oligonucleotides (ASOs), small interfering RNA (siRNA), short hairpin RNA (shRNA) [53], and microRNAs (miRs). Mattis et al. 2015 reported the ability of allele-specific ASOs to lower levels of mutant huntingtin expression and reverse BDNF-withdrawal induced toxicity in 109 and 180 CAG HD iPSC derived neurons [36]. As mentioned above, many existing iPSC lines are homozygous at SNPs that could be used for allele-specific targeting by known useful siRNAs, shRNAs, and miRs making allele specific knockdown of mutant huntingtin difficult or impossible using these methods. Zinc finger proteins (ZFPs) are also being developed (by Sangamo) to repress transcription of the HTT allele bearing expanded CAGs and thus specifically reduced mutant huntingtin protein levels.

CONCLUSIONS

Significant progress has been made in the HD iPSC field. Now that numerous HD iPSCs are available to researchers, individual studies should be performed using as many cell lines as appropriate based on a power analysis for individual phenotypes. The limited number of studies on iPSCs and their differentiated progeny using lines with adult onset CAG lengths is concerning since adult onset HD represents the vast majority of HD cases. Work with HD embryonic stem (ES) cells has focused on lower repeat lengths (37–51 CAG) [49, 143–145]. However, use of HD ES cells abrogates the ability to correlate cellular phenotypes with patient symptoms, a major effort underway in other neurodegenerative diseases using iPSC models [134]. Renewed efforts should be undertaken to continue to increase the repertoire of available stem cell lines as existing lines age with passage. Further investigations should be directed toward identifying phenotypes in HD iPSCs bearing an expanded allele in the adult onset range and investigating changes that occur in the absence of exogenous stress. New methods using 3-dimensional culture or co-culture systems may be key to unveiling phenotypes in the absence of stress. Finally, it would be helpful if the field as a whole would settle on one or two differentiation schemes to improve comparison among studies. Thanks to the pioneering work of numerous HD investigators, HD iPSCs are just beginning to show their promise and may be the key to finally identifying treatments useful in HD patients.

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CONFLICT OF INTERESTS

The authors have no conflicts to declare.

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