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Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line from a Huntington's disease patient with a long HTT-PolyQ sequence

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A B S T R A C T

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by an abnormal length of CAG repeats in the gene *HTT*, leading to an elongated polyglutamine (poly-Q) sequence in huntingtin (HTT). We used non-integrative Sendai virus to reprogram fibroblasts from a patient with juvenile onset HD to induced pluripotent stem cells (iPSCs). Reprogrammed iPSCs expressed pluripotency-associated markers, exhibited a normal karyotype, and following directed differentiation generated cell types belonging to the three germ layers. PCR analysis and sequencing confirmed the HD patient-derived iPSC line had one normal *HTT* allele and one with elongated CAG repeats, equivalent to ≥180Q.

Resource Table:

Unique stem cell line identifier	BIHi035-A https://hpscreg.eu/cell-line/BIHi035-A
Alternative name(s) of stem cell line	HD180
Institution	Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany; Heinrich Heine University, Düsseldorf, Germany
Contact information of distributor	Sebastian Diecke PhD, Technology Platform Pluripotent Stem Cells, Max Delbrück Center for Molecular Medicine (MDC), Haus 27, Robert-Rössle-Str. 10, 13125 Berlin, GERMANY T: +49 30 9406 3090 E: Sebastian.Diecke@mdc-berlin.de
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: ~6 years old Sex: male Ethnicity if known: Caucasian
Cell Source	Human Dermal Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai Cytotune 2.0
Genetic Modification	None
Type of Genetic Modification	–
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR from cDNA
Associated disease	Huntington's Disease
Gene/locus	Huntingtin (HTT), Chr4 (NC_000004.12 (3074681.0.3243960))
Date archived/stock date	2021
Cell line repository/bank	https://hpscreg.eu/cell-line/BIHi035-A
Ethical approval	The original fibroblast cell line (GM09197) was derived under informed consent of the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research, and obtained together with an MTA for research use.

1. Resource utility

Huntington's disease (HD) is a rare neurodegenerative disorder with suspected broader pathophysiology including cardiovascular dysfunction. Patient-derived iPSCs, in particular those with a severe *HTT* poly-CAG genotype, and their neuronal or cardiac derivatives, could provide

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast microscopy	Normal	Fig. 1A
Phenotype	Qualitative analysis: immunofluorescence microscopy	Expression of multiple pluripotency-associated factors	Fig. 1C
	Quantitative analysis: flow cytometry	High percentage of cells expressing pluripotency factors	Fig. 1D
Genotype	Karyotype (SNP microarray), 0.3 megabases	46 XY	Fig. 1B, supplementary Fig. S1A
Identity	STR analysis	10 specific sites tested, line matches patient-derived fibroblasts	Submitted in archive with journal
Mutation analysis	Mutation PCR	Confirmed the presence of elongated *HTT* allele	Fig. 1F
	Sequencing	Confirmed elongated poly-CAG repeats in *HTT*	Fig. 1G
Microbiology and virology	Mycoplasma qRT-PCR	Negative	Supplementary Fig. S1C
Differentiation potential	Flow cytometry (immunofluorescence) following lineage specific directed differentiation	Expression of lineage specific markers confirmed: SOX17 (endoderm), TNNT2 (mesoderm), PAX6 (ectoderm)	Fig. 1E
Donor screening	HIV 1 + 2, Hepatitis B, Hepatitis C	Negative	Not shown but available with author

advances in the mechanistic understanding of the disease pathogenesis and the discovery of potential treatment strategies (see Table 1).

2. Resource details

As part of a long term study into HD, we began reprogramming samples from multiple HD patients into iPSCs (Miller et al., 2022). In order to broaden this study and identify the pathophysiology arising from extremely elongated forms of the protein Huntingtin (HTT) forming extended glutamine stretches (poly-Q), we obtained human dermal fibroblasts from an anonymous male juvenile patient suffering early onset HD. Cells were reprogrammed to iPSCs using Sendai virus, with individual colonies isolated and outgrown under feeder-free pluripotent culture conditions. Following several passages one of the clones exhibiting stable and uniform iPSC morphology was selected for expansion and further characterisation (Fig. 1A). Single nucleotide polymorphism (SNP) microarray as late as passage 31 (P31) revealed a normal karyotype with no chromosome alterations (Fig. 1B), with several small variations marginally above cut-off of $3x10^5$ bases (O'Shea et al., 2020) (Fig. S1A). Analysis by reverse-transcription PCR (RT-PCR) at P28 indicated an absence of viral transgene expression (Fig. S1B). Cells were also negative for mycoplasma at P31 (Fig. S1C), as well as other human pathogenic viruses (data available upon request). STR profiling also confirmed that the donor fibroblasts and P31 reprogrammed iPSCs had matching identity (archived data). Following fixing and immunostaining, expression of pluripotency-associated transcription factors and cell surface markers was confirmed by immunofluorescence microscopy (P31) and flow cytometry (P29) analysis (Fig. 1C-D). Directed differentiation of BIHi035-A iPSCs produced cell types from all three germ layers, with robust expression of definitive endoderm, cardiomyocyte (mesoderm), and neuronal (ectoderm) markers detected (Fig. 1E).

To confirm the pathogenic poly-CAG genotype of *HTT*, PCR primers flanking exon 1 were used to amplify the region from fibroblast and iPSC genomic DNA (Fig. 1F). Sanger sequencing of excised bands confirmed the poly-CAG motif in both alleles (Fig. 1G, black arrows indicate 5′ end of reverse complement). Interestingly, the pathogenic larger band in derivative iPSCs had increased in length compared to the parental fibroblasts, from ~180 to ~195 poly-Q. This is in line with previously observed instability of very long *HTT* poly-CAG sequences in iPSC culture over time (Goold et al., 2019).

3. Materials and methods

3.1. iPSC reprogramming and maintenance

Human dermal fibroblasts at passage 11 were reprogrammed to iPSCs using Cytotune 2.0 Sendai virus (Thermo Fisher), according to manufacturer guidelines. Fibroblasts were plated onto 0.1 % gelatin-coated plates in DMEM (Thermo) containing 10 % foetal calf serum (Thermo) at $1–2 \times 10^4$ cells/cm^2 and cultured at 37 °C in 5 % CO_2. Two days later cells were transduced with a mixture of KOS, hcMyc and KLF4 viruses at respective MOIs of ~5:5:3, together with 5 µg/ml polybrene (Millipore), and thenceforth cultured at 37 °C in 5 % CO_2 and 5 % O_2 (hypoxia). After seven days cells were dissociated using TrypLE (Thermo) and replated onto Geltrex (Thermo) at $\sim 1 \times 10^3$ cells/cm^2. Next day and thenceforth E8 medium (Thermo) was used, with individual colonies manually picked and expanded after 2–3 weeks. Subsequent passaging was performed non-enzymatically in small clusters using PBS/EDTA, with Y-27632 (Tocris) ROCK-inhibition included at 1:1000 v/v on the day of passaging during the early stages and following thawing.

3.2. Immunostaining and microscopy

Cells were fixed with 4 % paraformaldehyde (Science Services) for 20 min at room temperature (RT), washed with PBS$^-$, and incubated with blocking solution containing 10 % normal goat serum (Abcam) and 0.1 % Triton X-100 (Sigma-Aldrich) in PBS$^-$ with 0.05 % Tween 20 (Sigma-Aldrich) for 1 h at RT. Primary antibodies were incubated overnight at 4 °C and secondary antibodies for 1 h at RT (see Table 2). Nuclei were counterstained with 1:10,000 Hoechst (Thermo). All microscopy images were acquired on a DMi8 microscope fitted with a K5 camera, with images processed using LASX software (all from Leica).

3.3. RT-PCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen). Mycoplasma testing was performed using the Venor®GeM qOneStep Kit according to manufacturer's instructions. Thermocycling and real-time analysis were performed using a QuantStudio 6 (Thermo). Sendai clearance was tested by RT-PCR following conversion of 1 µg RNA using iScript cDNA Synthesis Kit (BioRad). PCR was performed using polymerase KAPA2G Hotstart (KAPABiosystems) and primers listed in Table 2, in a SimpliAmp thermocycler (Thermo): initial denaturation 95 °C for 3 min,

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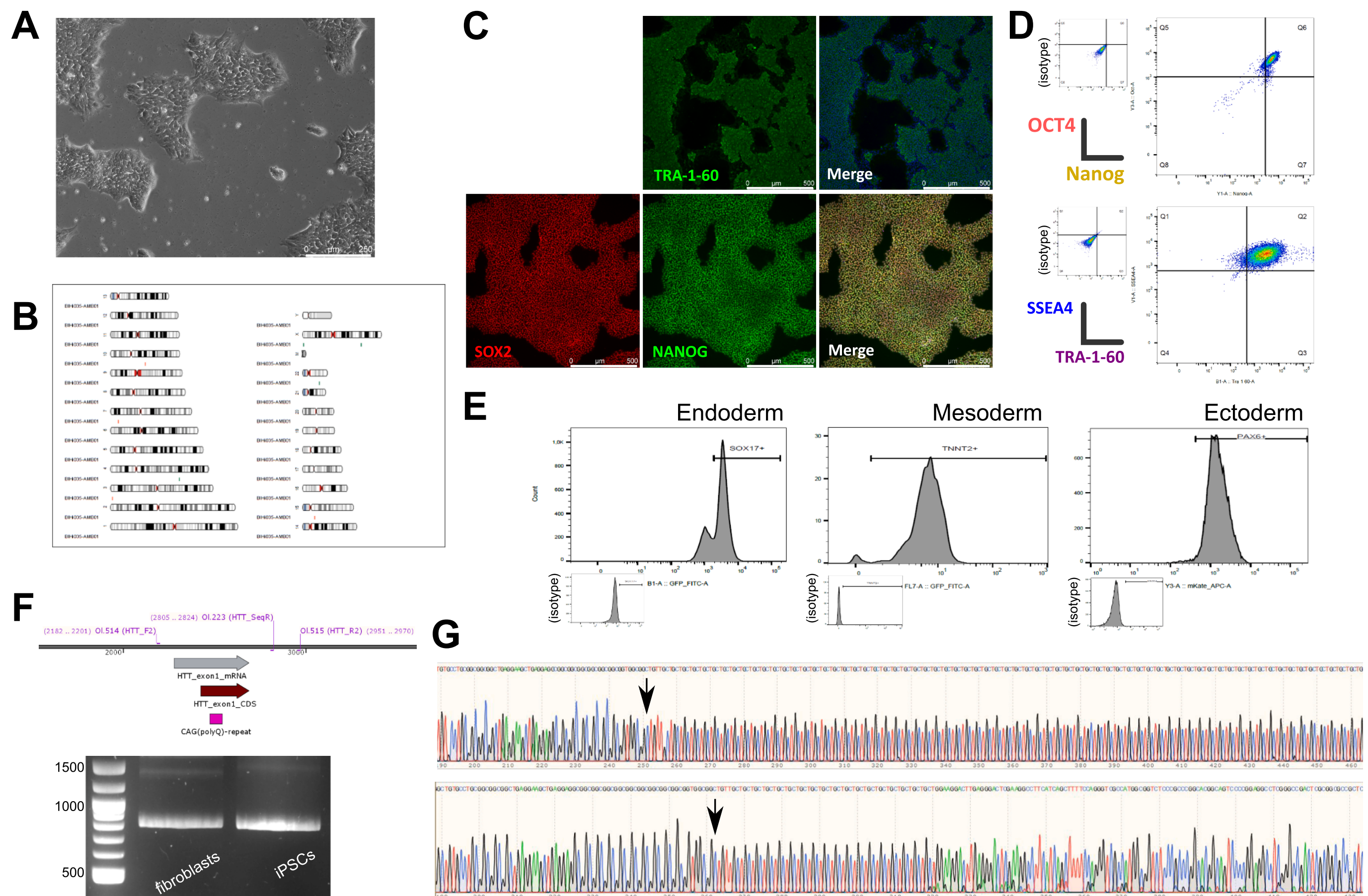

Fig. 1. Confirmation of pluripotency and *HTT*-elongated CAG in one iPSC line generated from a juvenile patient with Huntington's disease (HD).

30–40 cycles of [denaturation 95 °C for 15 s, annealing 55–65 °C for 15 s, extension 72 °C for 15–60 s/kb], final extension 72 °C for 1 min. Products were visualised by agarose gel electrophoresis.

3.4. Flow cytometry analysis

Cells were harvested using TrypLE, stained for viability using VioBility Blue (Miltenyi), fixed and permeabilised using FoxP3 staining buffer kit (Miltenyi), and stained with conjugated antibodies (Table 2). Expression was analysed using a MACSQuant VYB flow cytometer (Miltenyi) with gating and plots generated using FlowJo 10.

3.5. Directed differentiation

Differentiations were performed on Geltrex at 37 °C in 5 % CO_2 and 20 % O_2 (normoxia). For ectodermal differentiation, iPSCs were enzymatically dissociated and replated at 2×10^5 cells/well of a 12-well plate in ectoderm-specific medium from the StemMACS™ Trilineage differentiation kit (Miltenyi), followed by daily medium changes. For mesodermal differentiation, cells were specified to the cardiac lineage using an adapted version of an established differentiation protocol (Lian et al., 2012). Metabolic selection using sodium lactate (Sigma) was applied during days 10–12. For endodermal differentiation, iPSCs were dissociated and replated at 2×10^6 cells/well of a 6-well plate in pluripotency medium. Next day, medium was replaced with Medium 1 of StemMACS™ Definitive Endoderm differentiation kit (Miltenyi), and on subsequent days with Medium 2, according to manufacturer's instructions.

3.6. HTT genotyping

Genomic DNA was isolated with FlexiGene DNA Kit (QIAGEN). Exon 1 of the *HTT* locus was amplified by PCR using PrimeSTAR GXL polymerase and primers listed in Table 2. A SimpliAmp thermocycler was used: initial denaturation 98 °C for 2 min, 36 cycles of [denaturation 98 °C for 15 s, annealing 60 °C for 15 s, extension 68 °C for 1 min 30 s], final extension 68 °C for 2 min. For Sanger sequencing, PCR products were excised from a low-melt agarose gel (Carl Roth), purified and submitted to LGC Genomics. Chromatograms were analysed using SnapGene.

3.7. Karyotyping and STR

SNP karyotyping was assessed using Infinium OmniExpressExome-8 Kit and the iScan system from Illumina. Copy number variations (CNVs) and SNP visualizations were determined using KaryoStudio v1.3 (Illumina). For STR, ten microsatellite loci were amplified via PCR and labelled using the GenePrint® 10 system (Promega). Analysis was performed with ABI 3730xl DNA analyser (Thermo Fisher).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow cytometry				
	Antibody	RRID	Dilution	Company (Cat #)
Pluripotency Marker	Mouse monoclonal anti-TRA-1–60 IgM	AB_2119059	1:100	Cell Signaling (#4746)
Pluripotency Marker	Rabbit polyclonal anti-NANOG IgG	AB_2539867	1:100	Thermo Fisher (#PA1-097)
Pluripotency Marker	Mouse monoclonal anti-SOX2 IgG	AB_2536667	1:200	Thermo Fisher (MA1-014)
Pluripotency Marker FACS	OCT4-PE	AB_2653086	1:50	Miltenyi (130-120-310)
Pluripotency Marker FACS	Nanog-APC	AB_2652990	1:50	Miltenyi (130-105-079)
Pluripotency Marker FACS	SSEA4-VioBlue	AB_2653521	1:10	Miltenyi (130-098-366)
Pluripotency Marker FACS	TRA-1-60-FITC	AB_2654228	1:50	Miltenyi (130-106-872)
Differentiation Marker FACS	TNNT2-FITC	AB_2751735	1:50	Miltenyi (130-119-575)
Differentiation Marker FACS	SOX17-Vio®B515	AB_2653496	1:50	Miltenyi (130-111-147)
Differentiation Marker FACS	CXCR4-APC	AB_2752192	1:50	Miltenyi (130-120-778)
Differentiation Marker FACS	PAX6-APC	AB_2653168	1:10	Miltenyi (130-107-829)
Differentiation Marker FACS	REA-VioBlue (isotype control)	AB_2733972	1:50	Miltenyi (130-113-454)
Differentiation Marker FACS	REA-FITC (isotype control)	AB_2733688	1:50	Miltenyi (130-113-449)
Differentiation Marker FACS	REA-Vio®B515 (isotype control)	AB_2751239	1:50	Miltenyi (130-114-556)
Differentiation Marker FACS	REA-PE (isotype control)	AB_2751113	1:50	Miltenyi (130-113-462)
Differentiation Marker FACS	REA-APC (isotype control)	AB_2733446	1:50	Miltenyi (130-113-446)
Secondary antibody	Goat anti-rabbit IgG AF488	AB_2576217	1:500	Thermo Fisher (#A-11034)
Secondary antibody	Goat anti-rabbit IgG AF555	AB_2535844	1:500	Thermo Fisher (#A-21422)
Secondary antibody	Goat anti-mouse IgM AF488	AB_2535711	1:500	Thermo Fisher (#A-21042)

Primers			
	Target	Amplicon	Forward/Reverse primer (5′-3′)
Sendai virus (RT-PCR)	*SeV*	181 bp	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAGAGATATGTATC
Sendai virus (RT-PCR)	*KOS*	528 bp	F: ATGCACCGCTACGACGTGAGCGC R: ACCTTGACAATCCTGATGTGG
Sendai virus (RT-PCR)	*KLF4*	410 bp	F: TTCCTGCATGCCAGAGGAGCCC R: AATGTATCGAAGGTGCTCAA
Sendai virus (RT-PCR)	*cMyc*	532 bp	F: TAACTGACTAGCAGGCTTGTCG R: TCCACATACAGTCCTGGATGATGATG
Sendai virus (RT-PCR)	*18 s* (housekeeping)	151 bp	F: GTAACCCGTTGAACCCCATT R: CCATCCAATCGGTAGTAGCG
Mutation HTT (PCR)	*HTT*	~756–1320 bp	F2: GGCTAGGGCTGTCAATCATG R2: ACTCCCCTCGGTGAATTCAG SeqR: GTTGCTGGGTCACTCTGTCT

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103056.

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