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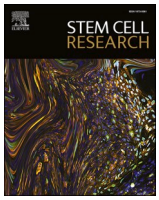

Lab Resource: Multiple Cell Lines

Generation of induced pluripotent stem cells from three individuals with Huntington's disease

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A B S T R A C T

Huntington's disease (HD) is a neurodegenerative disorder caused by abnormal glutamine (Q) expansion in the huntingtin protein due to elongated CAG repeats in the gene HTT. We used non-integrative episomal plasmids to generate induced pluripotent stem cells (iPSCs) from three individuals affected by HD: CH1 (58Q), and two twin brothers CH3 (44Q) and CH4 (44Q). The iPSC lines exhibited one healthy HTT allele and one with elongated CAG repeats, as confirmed by PCR and sequencing. All iPSC lines expressed pluripotency markers, exhibited a normal karyotype, and generated cells of the three germ layers *in vitro*.

1. Resource Table

Please fill in right-hand column of the table below. All information requested in the table is MANDATORY, except where otherwise indicated. Manuscripts with incomplete or incorrect information will be sent back to author.

Unique stem cell lines identifier	BIHi288-A BIHi033-A BIHi034-A
Alternative name(s) of stem cell lines	CH1 (BIHi288-A) CH3 (BIHi033-A) CH4 (BIHi034-A)
Institutions	Charité – Universitätsmedizin Berlin, Germany; Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany; Heinrich Heine University, Düsseldorf, Germany;
Contact information of distributor	

(*continued on next column*)

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Unique stem cell lines identifier	BIHi288-A BIHi033-A BIHi034-A
	Prof. Dr. Josef Priller, Neuropsychiatry & Laboratory of Molecular Psychiatry, Charité – Universitätsmedizin Berlin, Germany
Type of cell lines	iPSCs
Origin	Human
Additional origin info required *for human ESC or iPSC*	Age: 35–45, 35–45, 35–45 Sex: Male, Male, Male Ethnicity if known: Caucasian, Caucasian, Caucasian
Cell Source	Human dermal fibroblasts
Clonality	Clonal
Associated disease	Huntington's disease
Gene/locus	Huntingtin (HTT), Chr4 (NC_000004.12 (3074681.0.3243960))
Date archived/stock date	2019, 2021, 2022
Cell line repository/bank	

(*continued on next page*)

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(*continued*)

Unique stem cell lines identifier	BIHi288-A BIHi033-A BIHi034-A
	https://hpscreg.eu/cell-line/BIHi288-A https://hpscreg.eu/cell-line/BIHi033-A https://hpscreg.eu/cell-line/BIHi034-A
Ethical approval	The original study was approved by the IRB of the Charité – Universitätsmedizin Berlin (EA1/243/12). The work was also approved by the Ethic Committee of the Medical Faculty of Heinrich Heine University (study number: 2019–681). The lines cannot be freely shared under the current ethical approval.

2. Resource utility

Huntington's disease (HD) is a rare neurodegenerative disorder with potentially broader pathophysiology including cardiovascular changes, for which no therapy is available. The development of patient-derived iPSCs and their differentiation into specific somatic cell types could lead to advances in the mechanistic understanding of the disease pathogenesis and the discovery of potential treatment strategies.

3. Resource details

Huntington's disease (HD) is an untreatable neurodegenerative disorder caused by an abnormal expansion of a CAG repeat in the gene encoding the protein huntingtin (HTT) (Zuccato et al., 2010). This mutation results in an expanded glutamine (Q) stretch, which confers a pathogenic function to HTT when longer than 35 Q residues.

We obtained skin fibroblasts from three individuals carrying the HD mutation. To protect the anonymity of the patients, we only received the following information: gender, age range, and Q length. Case CH1 (Chorea Huntington 1) was male, 35–45 years-old, with 58Q. Cases CH3 (Chorea Huntington 3) and CH4 (Chorea Huntington 4) were twin brothers, 35–45 years-old, with 44Q (Scior et al., 2018). We generated induced pluripotent stem cells (iPSCs) from fibroblasts obtained by skin biopsies from the three individuals using non-integrative episomal plasmids containing the reprogramming factors OCT3/4, SOX2, c-MYC, LIN28 and KLF4 (Lorenz et al., 2017).

We obtained three iPSC lines: CH1, CH3, and CH4 (Table 1). STR analysis confirmed that the iPSC lines were derived from the relative patient fibroblasts. All iPSC lines displayed human pluripotent stem cell-like colony morphology and growth behaviour at passage 12 (Fig. 1**A,** scale bars: 100 μm). For all lines, we confirmed episomal vector dilution at passage 15 with RT-PCR showing absence of vector-derived exogenous *OCT4* (Fig. S1**A**). For CH1, expression of pluripotency markers NANOG and TRA-1–60 at the protein level was validated at passage 16 (Fig. 1**B,** scale bars: 100 μm). Using RT-qPCR with control iPSCs XM001 (Wang et al., 2018) as a standard, we confirmed that CH1 expressed pluripotency markers *NANOG, SOX2, DPPA4,* and *DNM3TB* at passage 18, with downregulation of the fibroblast marker vimentin (*VIM*) **(**Fig. 1**C).** For CH3 and CH4, we confirmed the expression of pluripotency-associated protein markers SOX2, NANOG, OCT4, SSEA4, and TRA-1–60 at passage 16 (Fig. 1**D**, scale bars: 100 μm). For CH1, we used *in vitro* embryoid body (EB)-based differentiation to validate its capacity to form cells of the three germ layers, as seen by expression of protein markers SOX17 and FOXA2 (endoderm), TUJ1 and NESTIN (ectoderm), and smooth muscle actin (SMA) and BRACHYURY (mesoderm) (Fig. 1**E**, scale bars: 100 μm). For CH3 and CH4, we used directed differentiation and confirmed the formation of each germ layer using fluorescence activated cell sorting (FACS) with markers CD140b and CD144 (mesoderm), CXCR4 and SOX17 (endoderm), and PAX6 and SOX2 (ectoderm) (Fig. 1**F**). All HD iPSC lines displayed normal karyotypes at passage 25 with no chromosome alterations, and only small heterozygous losses or gains, or loss-of-heterozygosity, well below a cut-off of $1x10^6$ bases **(**Fig. 1**G).**

To monitor and validate the presence of mutant *HTT*, we used PCR to amplify the *HTT* exon 1 region. Gel electrophoresis demonstrated that healthy iPSC line XM001 (Wang et al., 2018) showed only one broad band, indicating two *HTT* alleles within the normal range of 6–35 CAG repeats. Conversely, HD iPSC lines CH1, CH3, and CH4 each displayed two bands with one higher than the other, suggesting that one allele was elongated due to extended CAG repeats (Fig. 1**H**). Sanger sequencing analysis confirmed the presence of an allele with an extended CAG repeat in all three HD iPSC lines (Fig. 1**I,** black arrows indicate the 3′ end of the reverse complement of CAG repeats).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis: Immunocytochemistry	CH1: Positive for NANOG, TRA-1–60 CH3-4: Positive for SOX2, NANOG, OCT4, SSEA4, TRA-1–60	Fig. 1B, Fig. 1D
	Quantitative analysis: RT-qPCR	Positive NANOG, SOX2, DPPA4, DNMT3B	Fig. 1C
Genotype	SNP array Resolution: 0.3 megabases	*46XY* *46XY* *46XY*	Fig. 1G
Identity	STR analysis	10 specific sites tested Lines matched respective patient-derived fibroblasts	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Mutation PCR	Confirmed the presence of elongated alleles in the patient lines	Fig. 1H
	Sequencing	Confirmed elongated CAG repeats in the patient lines	Fig. 1I
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: negative	Fig. S1B
Differentiation potential	Embryoid body formation	For CH1: Immunostaining positive for SMA and BRACHYURY (mesoderm), SOX17 and AFP (endoderm), TUJ1 and NESTIN (ectoderm)	Fig. 1E
Differentiation potential	FACS	For CH3 and CH4: CD140b and CD144 (mesoderm), CXCR4 and SOX17 (endoderm), PAX6, SOX2 (ectoderm)	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2, Hepatitis B, Hepatitis C	Negative	Not shown but available with JP

4. Materials and methods

4.1. iPSC reprogramming

Fibroblasts were reprogrammed with episomal plasmids (Yu et al.,

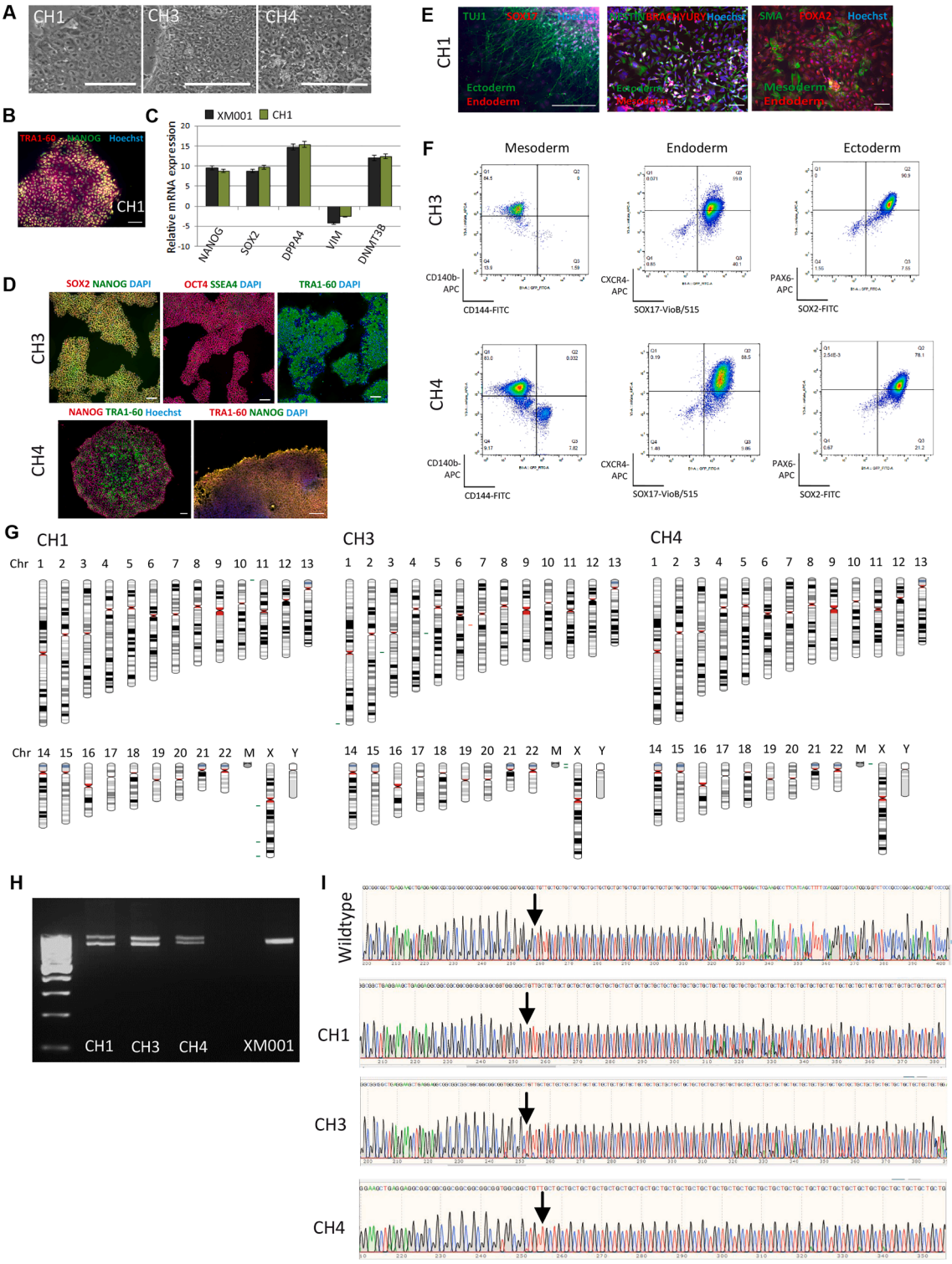

Fig. 1. Generation of three iPSC lines from three individuals with Huntingtoń s disease (HD).

2011) by nucleofecting oriP/EBNA-1- episomal plasmids expressing OCT3/4, SOX2, NANOG, KLF4, c-MYC and LIN28 using Amaxa Cell Line Nucleofector Kit R (Lonza). iPSCs were maintained in feeder-free conditions with StemMACS iPS-Brew XF (Miltenyi Biotec) and MycoZap in humidified atmosphere of 5 % CO_2 at 37 °C and 5 % oxygen. Exclusion of mycoplasma DNA was performed by PCR analysis (Fig. S1**B**).

4.2. Directed and non-directed differentiation

Directed differentiation was performed using the StemMACS™ Trilineage differentiation kit (Miltenyi Biotec). iPSCs were enzymatically dissociated to single cells and replated for six days in lineage-specific medium containing 10 μM Y-27632 (Tocris) between 0.5 and 2 × 10ˆ6 cells/well. For non-directed differentiation, EBs were grown for 1 week in non-adherent conditions, followed by adherence for ten days using KO-DMEM medium and 20 % knock-out serum replacement (Thermo).

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	RRID	Dilution	Company Cat # and RRID
Pluripotency Marker	Rabbit polyclonal anti-OCT-3/4 (H-134)	AB_2167703	1:100	Santa Cruz Cat# sc-9081
Pluripotency Marker	Mouse monoclonal anti-TRA-1–60	AB_2119059	1:100	Cell Signaling (#4746)
Pluripotency Marker	Rabbit polyclonal anti-NANOG	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	Mouse monoclonal anti-SSEA4	AB_1264259	1:100	Cell Signaling (#4755)
Differentiation Marker FACS	CD140b-APC	AB_2655084	1:50	Miltenyi (130–105-322)
Differentiation Marker FACS	CD144-FITC	AB_2655150	1:10	Miltenyi (130–100-713)
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	SOX2-FITC	AB_2653499	1:10	Miltenyi (130–104-993)
Differentiation Marker	Mouse monoclonal anti-SMA, clone 1A4	AB_2223500	1:200	DakoCytomation Cat# M0851
Differentiation Marker	Mouse monoclonal anti-β-Tubulin III (TUJ1)	AB_1841228	1:2000	Sigma-Aldrich Cat# T8578
Differentiation Marker	Anti-Nestin, clone 10C2	AB_2251134	1:200	Millipore Cat# MAB5326
Differentiation Marker	Goat polyclonal anti-SOX17	AB_355060	1:50	R&D Systems Cat#AF1924
Differentiation Marker	Goat polyclonal anti-BRACHYURY	AB_2200235	1:500	R&D Systems Cat#AF2085
Differentiation Marker	Rabbit polyclonal anti-FOXA2	AB_451718	1:100	Sevenhills Bioreagents Cat# WRAB-1200
Secondary antibody	Cy5-conjugated AffiniPure Goat anti-Rabbit IgG (H + L)	AB_2338013	1:300	Jackson Immuno Research Cat# 111–175-144
Secondary antibody	Cy3-conjugated Donkey anti-Mouse	AB_92642	1:300	Merck Millipore Cat# AP192C
Secondary antibody	Cy5-conjugated AffiniPure Donkey anti-Goat IgG (H + L)	AB_2340415	1:300	Jackson Immuno Research Cat# 705–175-147
Primers				
	Target	**Forward/Reverse primer (5′-3′)**		
Pluripotency Marker (qPCR)	NANOG	F: CCTGTGATTTGTGGGCCTG		
		R: GACAGTCTCCGTGTGAGGCAT		
Pluripotency Markers(qPCR)	SOX2	F: GTATCAGGAGTTGTCAAGGCAGAG		
		R: TCCTAGTCTTAAAGAGGCAGCAAAC		
Pluripotency Marker (qPCR)	DPPA4	F: TGGTGTCAGGTGGTGTGTGG		
		R: CCAGGCTTGACCAGCATGAA		
Pluripotency Marker (qPCR)	DNMT3B	F: GCTCACAGGGCCCGATACTT		
		R: GCAGTCCTGCAGCTCGAGTTTA		
Differentiation Marker (qPCR)	VIM	F: GGAGCTGCAGGAGCTGAATG		
		R: GACTTGCCTTGGCCCTTGAG		
House-Keeping Genes (qPCR)	ACTB	F: TCAAGATCATTGCTCCTCCTGAG		
		R: ACATCTGCTGGAAGGTGGACA		
House-Keeping Genes (qPCR)	GAPDH	F: CTGGTAAAGTGGATATTGTTGCCAT		
		R: TGGAATCATATTGGAACATGTAAACC		
Mycoplasma test (PCR)	Myco-f1	F: CGCCTGAGTAGTACGTTCGC		
Mycoplasma test (PCR)	Myco-f2	F: CGCCTGAGTAGTACGTACGC		
Mycoplasma test (PCR)	Myco-f3	F: TGCCTGAGTAGTCACTTCGC		
Mycoplasma test (PCR)	Myco-f4	F: CGCCTGGGTAGTACATTCGC		
Mycoplasma test (PCR)	Myco-f5	F: CGCCTGAGTAGTAGTCTCGC		
Mycoplasma test (PCR)	Myco-f6	F: TGCCTGGGTAGTACATTCGC		
Mycoplasma test (PCR)	Myco-r1	R: GCGGTGTGTACAAGACCCGA		
Mycoplasma test (PCR)	Myco-r2	R: GCGGTGTGTACAAAACCCGA		
Mycoplasma test (PCR)	Myco-r3	R: GCGGTGTGTACAAACCCCGA		
Episomal Plasmids (RT-PCR)	OCT4 Plasmid	F: AGTGAGAGGCAACCTGGAGA		
		R: AGGAACTGCTTCCTTCACGA		
Endogenous OCT (RT-PCR)	OCT4 endogenous	F: GTGGAGGAAGCTGACAACAA		
		R: ATTCTCCAGGTTGCCTCTCA		
Mutation HTT (PCR)	HTT	F: GGCTAGGGCTGTCAATCATG		
		R: ACTCCCCTCGGTGAATTCAG		
		Seq.: GTTGCTGGGTCACTCTGTCT		

4.3. Immunostaining

Cells were fixed with 4 % paraformaldehyde (Science Services) for 20 mins at room temperature (RT), washed with PBS, and incubated with blocking solution containing 10 % normal donkey serum (Abcam) and 0.1 % Triton X-100 (Sigma-Aldrich) in PBS with 0.05 % Tween 20 (Sigma-Aldrich) for 1 hr at RT. Primary antibodies were incubated overnight at 4 °C and secondary antibodies for 1 hr at RT. Nuclei were counterstained with 1:10,000 Hoechst (Thermo). Images were acquired with AxioVision V4.6.3.0 software (Zeiss) and ImageJ.

4.4. RT-qPCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen). Gene expression analysis was performed with SYBR Green PCR Master Mix and ViiA 7 Real-Time PCR (Applied Biosystems) with $2^{-\Delta\Delta CT}$ and normalization with *ACTB* - *GAPDH*. Data are presented as mean log2 ratios in relation to control iPSCs XM001 (Wang et al., 2018). Lack of integration of episomal plasmids was assessed by RT-PCR. Vector pEP4 E02S ET2K was used as positive control. PCR conditions were: 32 cycles and each cycle contained 94 °C for 15 s, 58 °C for 30 s, and 68 °C for 1 min.

4.5. FACS analysis

Cells were harvested using 1x TrypLE (Gibco), 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 data gated and plotted using FlowJo 10 software.

4.6. HTT mutation analysis

Genomic DNA was isolated with FlexiGene DNA Kit (QIAGEN). Exon 1 of the *HTT* locus was amplified by PCR using PrimeSTAR GXL polymerase and specific primers (Table 2). Products were visualised by agarose gel electrophoresis. For Sanger sequencing, *HTT* PCR products were run on a low-melt agarose gel (Carl Roth), individual DNA bands were excised and purified using a gel extraction kit (Qiagen), and submitted to LGC Genomics. Chromatograms were analysed using SnapGene.

4.7. Karyotyping and STR

SNP karyotyping was assessed using Infinium OmniExpressExome-8 Kit and the iScan system from Illumina. CNV and SNP visualization were performed using KaryoStudio v1.4 (Illumina). For STR, 10 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.

Acknowledgements

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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.2022.102976.

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