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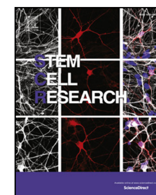

Lab resource: Stem cell line

Generation of the induced pluripotent stem cell line CSSi006-A (3681) from a patient affected by advanced-stage Juvenile Onset Huntington's Disease

Giovannina Rotundo[a,1], Eris Bidollari[a,1], Daniela Ferrari[b], Iolanda Spasari[c], Laura Bernardini[c], Federica Consoli[d], Alessandro De Luca[d], Iolanda Santimone[e], Giuseppe Lamorte[c], Simone Migliore[e], Ferdinando Squitieri[e], Angelo Luigi Vescovi[a], Jessica Rosati[a,*]

[a] *IRCCS Casa Sollievo della Sofferenza, Cellular Reprogramming Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy*
[b] *Biotechnology and Bioscience Department Bicocca University, Piazza della Scienza 2, Milan 20126, Italy*
[c] *IRCCS Casa Sollievo della Sofferenza, Cytogenetic Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy*
[d] *IRCCS Casa Sollievo della Sofferenza, Molecular Genetics Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy*
[e] *IRCCS Casa Sollievo della Sofferenza, Huntington and Rare Disease Unit, Viale dei Cappuccini, San Giovanni Rotondo, Foggia 71013, Italy*

A B S T R A C T

Juvenile Onset Huntington's Disease (JOHD) is a rare variant of HD withage of onset ≤20 years, accounting for 3–10% of all HD patients. The rarity occurrence of JOHD cases, who severely progress towards mental and physical disability with atypical clinical manifestations compared to classical HD, are responsible of general lack of knowledge about this variant. We obtained a fully reprogrammed iPS cell line from fibroblasts of a JOHD patient carrying 65 CAG repeats and age at onset at age 15. At the biopsy time, the patient showed an advanced stage after 10 years of disease.

Resource table

Unique stem cell line identifier	*CSSi006-A (3681)*
Alternative name(s) of stem cell line	*HD438.01 Cl.C*
Institution	*Cellular Reprogramming Unit, IRCCS Casa Sollievo della Sofferenza, Viale dei Cappuccini, San Giovanni Rotondo, Foggia Italy*
Contact information of distributor	*Jessica Rosati,j.rosati@css-mendel.it*
Type of cell line	*iPSC*
Origin	human
Additional origin info	Age:25 Sex:F Ethnicity: Caucasian
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Non integrating episomal vectors
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Huntington's Disease
Gene/locus	IT15/HD GENE- 4p16.3
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	April 2017
Cell line repository/bank	N/A
Ethical approval	Fondazione Casa Sollievo della Sofferenza Ethics Committee, approval number 75/CE

Resource utility

To date, there is no effective disease-modifying treatment for JOHD because its rarity and because symptomatology is complex and differs

* Corresponding author.
E-mail address: j.rosati@css-mendel.it (J. Rosati).
[1] These two authors contribute equally to the paper

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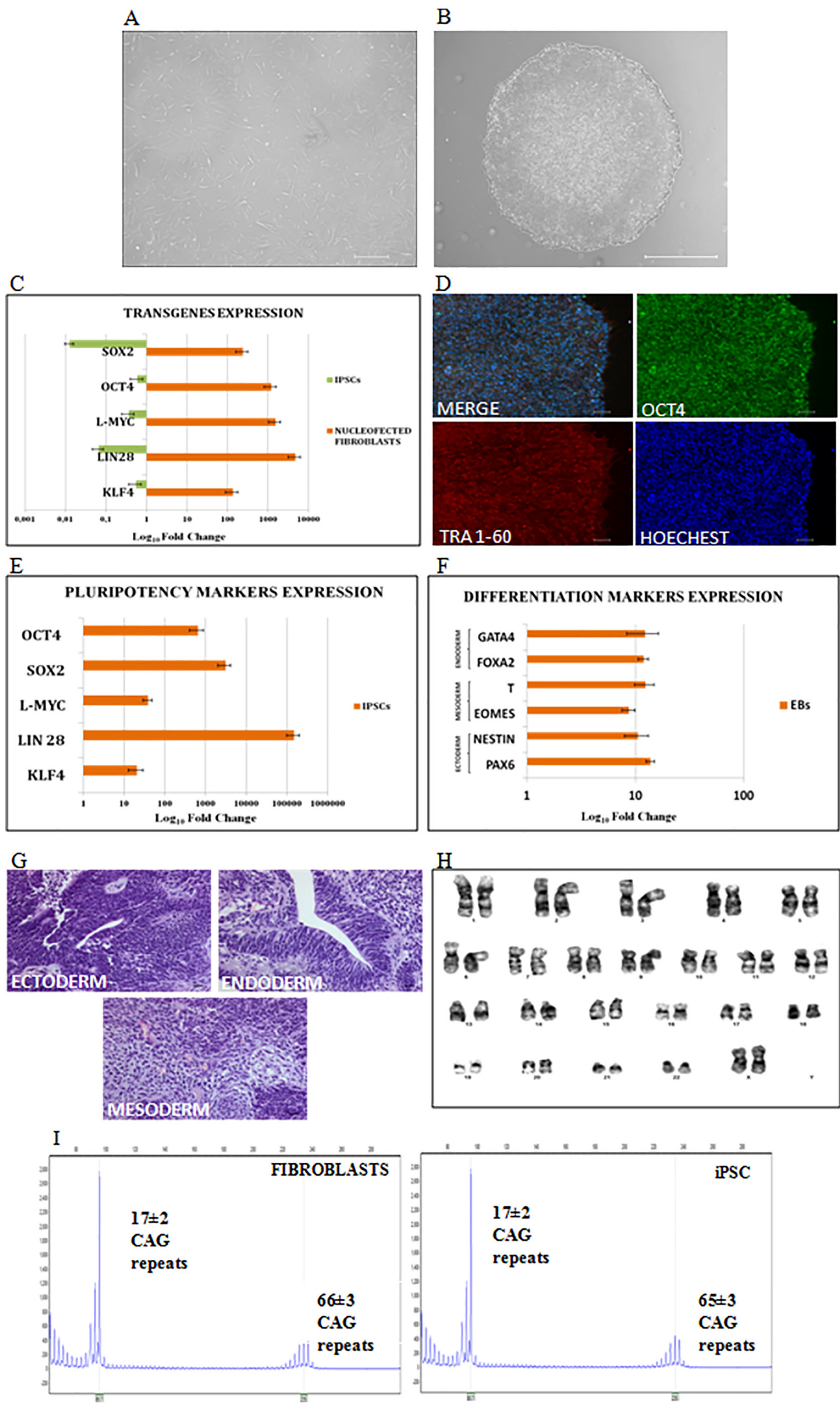

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Fig 1. A, B. Representative transmitted light images showing fibroblasts and induced pluripotent stem cell morphologies, scale bars correspond to 100 μm; C. Real time PCR analysis of transgenes expression; histograms show the fold change of transgenic transcript levels of KLF4, LIN28, L-MYC, OCT4, SOX2 in iPSC and nucleofected fibroblasts, using 18S as references; D. Immunofluorescence analysis of pluripotency markers OCT4 and TRA1-60 in iPSC, scale bars correspond to 100 μm; E. Real time PCR analysis of pluripotency markers; histograms show the fold change of endogenous transcripts levels of KLF4, LIN28, L-MYC, OCT4, SOX2 in iPSC and fibroblasts, using 18S as references; F. Real time PCR analysis of differentiation markers; histograms show the fold change of endogenous levels of NESTIN, PAX6, FOXA2, GATA4, T, EOMES in iPSC and in embryoid bodies, using 18S as references; G. Histological analysis of teratoma; derivatives of all three germ layers were observed; H. Karyogram displaying a normal 46 XX karyotype without any measurable anomalies; I. Chain reaction (PCR) results showing allele size at the HD CAG (n) repeat region in fibroblast and IPS cell line. PCR results with 17 ± 2 and 66 ± 3 CAG repeats in fibroblast and 17 ± 2 and 65 ± 3 CAG repeats in IPS cell lines.

from classical HD, which has been more thoroughly studied (Quarrell et al., 2009). The production of this JOHD-iPS line may contribute to add knowledge to the pathogenesis of this disease.

Resource details

Our patient is a 25-year-old woman carrying 16 and 65 CAG repeats, who scored a Total Functional Capacity value of 3 (The TFC scale ranges from 13 (normal) to 0 (severe disability)) when skin biopsy was performed (Marder et al., 2000). She is the only daughter of a non-consanguineous couple with family history for Huntington's disease, with paternal inheritance. Her development was normal until 12 years of age, when she suddenly developed episodes of obsessive thoughts and repetitive behaviour. During the following years, her parents noticed progressive and disabling dystonia starting at age 15, followed by disabling motor disorders, marked rigidity, inability to ambulate, mood disorders and numerous attempts at suicide. Skin fibroblasts (Fig. 1A), obtained from biopsy, were reprogrammed into pluripotent stem cells through nucleofection of episomal vectors expressing the Yamanaka factors: OCT4, KLF4, L-MYC, SOX2, LIN28 and sh-p53 (Okita et al., 2011) (Table 1). Cells were grown in xeno-free medium, under feeder-free conditions. The iPSC line showed typical human stem cell-like morphology with well-defined and flattened cell colonies (Fig. 1B). Non-integrative episomal vectors, used for the nucleofection, were progressively lost from the cells and, after ten passages, iPSC colonies did not express transgenes; the RNA extracted from nucleofected fibroblasts was used as a positive control of transgene expression (Fig. 1C). Pluripotency of this cell line was first confirmed by the expression of transcription factor OCT4 and of surface marker TRA-1-60 obtained through immunofluorescence analysis (Fig. 1D). Subsequently, a quantitative real-time PCR (qPCR) demonstrated expression levels of five pluripotent markers: OCT4, KLF4, LIN28, L-MYC, SOX2. Their expression appeared very high in the iPSC line and absent in the parental fibroblasts used as controls (Fig. 1E). The iPSC line produced embryoid bodies which spontaneously differentiated into three germ layers, whose expression was analysed through qPCR. Ectodermal lineage was confirmed by overexpression of NESTIN and PAX6, the mesodermal lineage was determined through expressions of Brachyury (T) and EOMES and the endoderm layer by the overexpression of GATA4 and FOXA2. iPSC line was used as normalizer (Fig. 1F). Differentiation capacity into three germ layers was also demonstrated through in vivo teratoma formation (Fig. 1G). Karyotype analysis demonstrated no accumulated chromosomal aberrations (Fig. 1H). We used polymerase chain reaction (PCR) to show allele size at the HD CAG $_{(n)}$ repeat region in fibroblast and IPS cell line; the results were 17 ± 2 and 65 ± 3 CAG repeats in fibroblasts and 17 ± 2 and 65 ± 3 CAG repeats in IPS (Fig. 1I). The identity/origin of this cell line was verified using short tandem repeats analysis (STR data available from the authors).

Materials and methods

Skin biopsy and fibroblast reprogramming

The skin biopsy was mechanically processed and maintained in fibroblast medium (DMEM high glucose, 20% FBS, 2 mM L-glutamine and 1% penicillin–streptomycin (all reagents from Sigma Aldrich)). Subsequently, 1×10^5 fibroblasts were nucleofected with 3 μg 1:1:1 mix of the episomal plasmids pCXLE-hUL (Addgene #27080), pCXLE-hSK (Addgene #27078) and pCXLE-hOCT4-shp53 (Addgene #27077). On day 7, the nucleofected fibroblasts were counted, plated on Matrigel (1:100) (BD Biosciences) and cultured in NutristemXF medium (Biological Industries). When iPSC colonies appeared, they were manually cut and amplified. PCR analysis, using EZ-PCR kit (Biological Industries), showed the absence of mycoplasma contamination.

Immunofluorescence staining

iPSC colonies were fixed with 4% paraformaldehyde for 20 min at room temperature. The cells were incubated with Blocking Buffer (PBS, 20% Normal Goat Serum, 0.1% Triton X-100) for 45 min at room temperature. Next, primary antibodies diluted in BSA 5% were

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	The cell line shows a normal iPSCs morphology	1B
Phenotype	Qualitative analysis Immunocytochemistry	The cell line shows expression for Pluripotency markers: Oct4, Tra1-60	1D
	Quantitative analysis RT-qPCR	The cell line shows expression for Pluripotency markers: OCT4, LIN28, L-MYC, KLF4, SOX2. Silenced transgene expression (from episomes): OCT4, LIN28, L-MYC, KLF4, SOX2	1C,1E
Genotype	Karyotype (G-banding) and resolution	Normal Karyotype: 46 XX, resolution: 450–500	1H
Identity	STR analysis	Tested 19 sites: all matched	Data is available from the authors
Mutation analysis (if applicable)	Sequencing	PCR results: 17 ± 2 and 46 ± 3 CAG repeats in fibroblasts 17 ± 2 and 65 ± 3 CAG repeats in IPS cell line	1I
Microbiology and virology	Mycoplasma	Mycoplasma tested by PCR: negative	Supplementary Fig. 1
Differentiation potential	Embryoid body formation and Teratoma formation	Genes expressed in embryod bodies: PAX6,NESTIN, EOMES, T, FOXA2,GATA4 Teratoma: proof of three germ layers formation	1F,1G

incubated overnight at 4 °C. After washing, Alexa Fluor 594- and/or Alexa Fluor 488-conjugated secondary antibodies in BSA 5% were added 1 h at room temperature. Nuclei were counterstained with DAPI. Micrographs were taken using a Nikon C2 fluorescence microscope and NIS Elements 1.49 software.

In vitro spontaneous differentiation

Mechanically detached iPSC were plated in Petri dishes in NutristemXF medium, which was substituted with differentiation medium: DMEM/F12, 20% KOSR (Gibco), 0,1 mM NEAA, 0.1 mM β-mercaptoethanol, 1% Pen/Strep the following day. The embryoid bodies (EBs) were maintained for 14 days.

Real-time PCR analysis

Trizol reagent (Life Technologies) was used to isolate total RNA from fibroblasts and iPSC. 1 μg of RNA, was reverse transcripted using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Each cDNA sample was tested in duplicate, 18S was used to normalize transcript abundance and calculations were performed with the delta Ct method. Primers are listed in Table 2.

Karyotyping

iPSCs were cultured in chamber slides (Thermo Fisher Scientific) coated with Matrigel in NutristemXF medium for 2–3 days. Cells were treated with a 0.1 μg/ml COLCEMID solution (Thermo Fisher Scientific) for 60 min at 37 °C. Metaphases were obtained by adding 30 mM KCl in 10%FBS at 37 °C for 6 min and by fixation, using a cold fresh-made 3:1 ethanol:acetic acid solution. Karyotype analysis was carried out on GTG-banded metaphases. Fifteen metaphases were counted and three karyotypes analysed. Only clonal aberrations were considered, following ISCN recommendations.

STR analysis

Fibroblasts and iPSCs DNA were extracted by Dneasy blood and tissue kit (QIAGEN). PCR amplification of 19 distinct STRs (D13S252, D13S305, D13S634, D13S800, D13S628, D18S819, D18S535, D18S978, D18S386, D18S390, D21S11, D21S1437, D21S1409, D21S1442, D21S1435, D21S1446, DXS6803, XHPRT, DXS1187) was carried out using the QST*Rplusv2 kit (Elucigene Diagnostics), PCR products were separated on an ABI Prism 3130 DNA sequencer and analysed by GeneMapper version 4.0 (Applied Biosystems).

Teratoma formation

Approximately 3×10^6 dispase-treated iPSCs, in 100 μl of Matrigel, were injected into nude mice, following ethical guidelines. About 4–6 weeks after injection, tumours were dissected, fixed in 10% formalin (Sigma Aldrich), paraffin-embedded, sectioned and stained with

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:100	Life Technologies (A13998)
	Mouse anti-TRA-1-60	1:100	Life Technologies (411000)
Secondary antibodies	anti-Rabbit AlexaFluor 488	1:1000	Invitrogen (A11034)
	anti-Mouse AlexaFluor 555	1:1000	Invitrogen (A21422)

Primers

	Target	Forward/Reverse primer (5′-3′)
Episomal genes	*eOCT4*	*Fwd: CAT TCA AAC TGA GGT AAG GG* *Rev: TAG CGT AAA AGG AGC AAC ATA G*
	eKLF4	*Fwd: CCA CCT CGC CTT ACA CAT GAAGA* *Rev: TAG CGT AAA AGG AGC AAC ATA G*
	eLIN28	*Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C* *Rev.: TAG CGT AAA AGG AGC AAC ATA G*
	eL-MYC	*Fwd: GGC TGA GAA GAG GAT GGC TAC* *Rev: TTT GTT TGA CAG GAG CGA CAA T*
	eSOX2	*Fwd: TTC ACA TGT CCC AGC ACT ACC AGA* *Rev: TTT GTT TGA CAG GAG CGA CAA T*
Pluripotency genes	*OCT4*	*Fwd: CCC CAG GGC CCC ATT TTG GTA CC* *Rev: ACC TCA GTT TGA ATG CAT GGG AGA GC*
	KLF	*Fwd: ACC CAT CCT TCC TGC CCG ATC AGA* *Rev: TTG GTA ATG GAG CGG CGG GAC TTG*
	LIN28	*Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C* *Rev: TCA ATT CTG TGC CTC CGG GAG CAGGGT AGG*
	L-MYC	*Fwd: GCG AAC CCA AGA CCC AGG CCT GCT CC* *Rev: CAG GGG GTC TGC TCG CAC CGT GAT G*
	SOX2	*Fwd: TTC ACA TGT CCC AGC ACT ACC AGA* *Rev: TCA CAT GTG TGA GAG GGG CAG TGT GC*
House-Keeping Genes	*18S*	*Fwd: GGC CCT GTA ATT GGA ATG AGT C* *Rev: CCA AGA TCC AAC TAC GAG CTT*
Differentiation Markers	*NESTIN*	*Hs04187831_g1*
	PAX6	*Hs00240871_m1*
	EOMES	*Hs00172872_m1*
	T	*Hs00610080_m1*
	GATA4	*Hs00171403_m1*
	FOXA2	*Hs00232764_m1*
	18S	*Hs03003631_g1*

hematoxylin/eosin. The presence of the three embryonic germ layers was analysed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.04.008.

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