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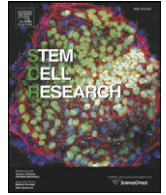
Lab resource: Stem cell line

Generation of spinocerebellar ataxia type 2 patient-derived iPSC line H271

Adele G. Marthaler [a,b,*,1], Alisa Tubsuwan [c,d,1], Benjamin Schmid [c], Ulla B. Poulsen [c], Poul Hyttel [a], Jørgen E. Nielsen [b], Troels T. Nielsen [b], Bjørn Holst [c]

[a] Department of Clinical and Veterinary Animal Science, Copenhagen University, Grønnegårdsvej 7, 1870 Frederiksberg, C, Denmark
[b] Neurogenetic Research Laboratory, Danish Dementia Research Centre, Department of Neurology, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark
[c] Bioneer A/S, Kogle Allé 2, 2970 Hørsholm, Denmark
[d] Stem Cell Research Group, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom 73170, Thailand

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ABSTRACT

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disease primarily affecting the cerebellum. Very little is known about the molecular mechanisms underlying the disease and, to date, no cure or treatment is available. Here, we demonstrate the generation of an induced pluripotent stem cell (iPSC) line of a SCA2 patient. The selected clone has been proven to be a *bona fide* iPSC line, which retains a normal karyotype. Due to its differentiation potential into neurons, this iPSC line will be a valuable tool in studying a disease-specific phenotype of SCA2.

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1. Resource table

Name of stem cell construct	H271 clone 1
Institution	University of Copenhagen and Bioneer A/S
Person who created resource	Adele G. Marthaler, Alisa Tubsuwan
Contact person and email	Adele G. Marthaler, adele.marthaler@sund.ku.dk
Date archived/stock date	October 2014
Origin	Human skin fibroblasts
Type of resource	Induced pluripotent stem cells; derived from skin fibroblasts of patient with spinocerebellar ataxia type 2
Sub-type	Cell line
Key transcription factors	Episomal plasmids containing *hOCT4, hSOX2, hL-MYC, hKLF4, hLIN28,* and *shP53* (Addgene plasmids 27077, 27078 and 27080; Okita et al., 2011)
Authentication	Identity and purity of stem cell line confirmed (Fig. 1)
Link to related literature (direct URL links and full references)	
Information in public databases	

2. Resource details

Human skin fibroblasts, obtained by skin biopsy of a symptomatic, male 33-year-old spinocerebellar type 2 (SCA2) patient (anonymized as H271), were reprogrammed using episomal vectors carrying transcripts for human *OCT4, SOX2, KLF4, L-MYC, LIN28,* and small hairpin RNA for *TP53* (Okita et al., 2011). The clone described in this publication was termed H271 clone (c) 1. The absence of the reprogramming plasmids was confirmed by quantitative PCR (qPCR) on genomic DNA (Fig. 1A).

SCA2 is a dominantly inherited neurodegenerative disorder caused by a mutation in the *ATXN2* gene. Normal alleles contain 22 CAG repeats with CAA interruptions (also coding for glutamine), whereas disease causing alleles contain trinucleotide repeats of 33 or more CAGs (usually without any CAA interruptions) (Pulst et al., 1996). The repeat lengths for patient H271 were determined to be 22 and 44 by fragment length analysis (data not shown) and confirmed to be present also in iPSC line H271 c1 by sequencing both alleles individually (Fig. 1B).

Furthermore, the expression of key pluripotency genes was observed both on RNA, as well as protein level, as demonstrated by qRTPCR analysis and immunocytochemistry, respectively (Fig. 1C and D). Additionally, the cells had the capacity to form derivatives of all three germ layers upon embryoid body differentiation (Fig. 1E). Taken together, this validates the true pluripotent potential of the generated iPSC line.
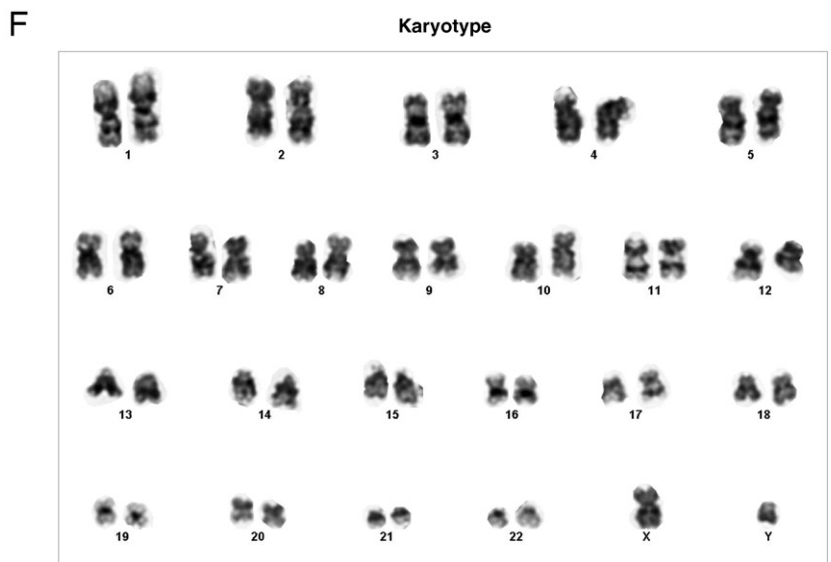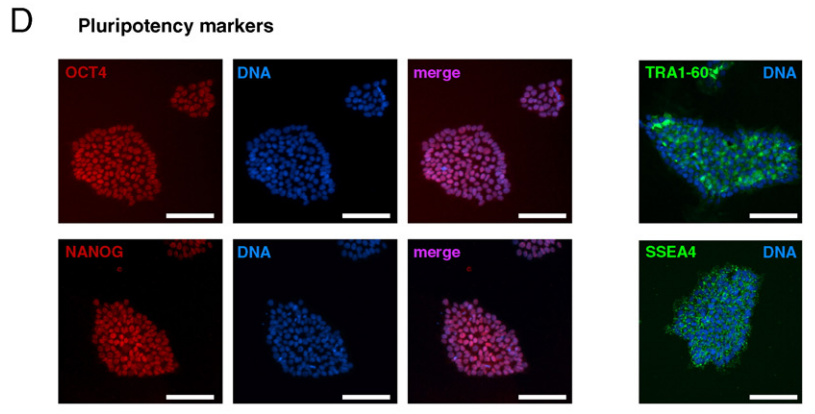
3. Materials and methods

3.1. Reprogramming using episomal vectors

Fibroblasts were cultured in fibroblast medium, consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10%

* Corresponding author at: Department of Clinical and Veterinary Animal Science, Copenhagen University, Grønnegårdsvej 7, 1870 Frederiksberg, C, Denmark.
E-mail address: adele.marthaler@sund.ku.dk (A.G. Marthaler).
[1] These authors contributed equally to this work.

A. Integration of reprogramming plasmids

B. Sequence of the expanded *ATXN2* allele

Sequence of the normal *ATXN2* allele

C. Pluripotency gene expression

D. Pluripotency markers

E. Ectoderm / Mesoderm / Endoderm

F. Karyotype
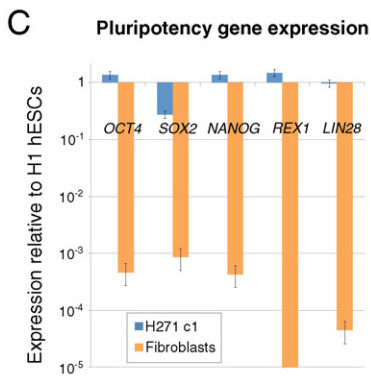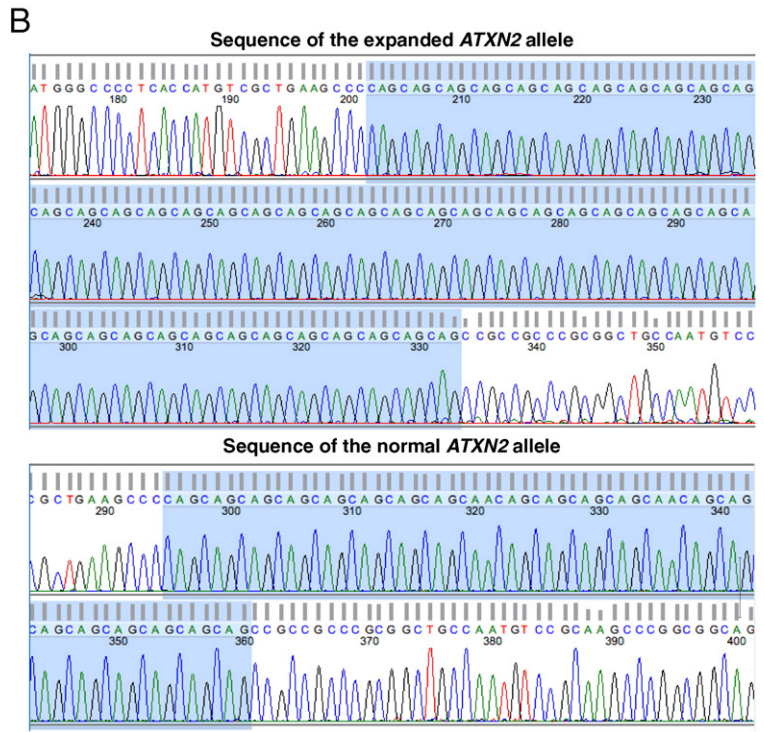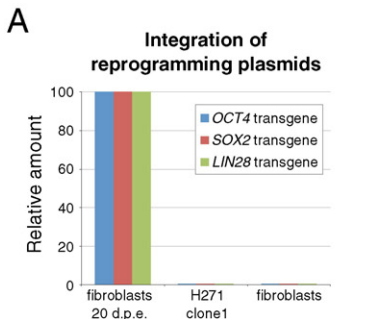
fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin and streptomycin (Pen/Strep). 1×10^5 fibroblasts were electroporated with a total of 1 μg of episomal plasmids containing *hOCT4* with a short hairpin to *TP53* (*shP53*; Addgene plasmids 27077 and 27076, respectively), *hSOX2* and *hKLF4* (Addgene plasmid 27078), and *hL-MYC* and *hLIN28* (Addgene plasmid 27080), (Okita et al., 2011), and cultured in fibroblast medium. Electroporation was carried out using a Neon™ electroporation device with two pulses at 1200 V for 20 ms (Life Technologies). One week after electroporation, fibroblasts were trypsinized and split 1:2 onto matrigel-coated dishes (BD Biosciences) and cultured in E8 medium (Gibco) under hypoxic conditions. After four weeks, primary iPSC colonies were picked to establish stable clones.

3.2. Integration of episomal vectors

DNA for integration analysis was purified from fibroblasts and iPSCs using the DNeasy Blood and Tissue kit (QIAGEN). Data was plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$ with fibroblasts and *GAPDH* as references. The following primers were used:

OCT4 transgene for	5′-CATTCAAACTGAGGTAAGGG-3′
OCT4 transgene rev	5′-TAGCGTAAAAGGAGCAACATAG-3′
SOX2 transgene for	5′-TTCACATGTCCCAGCACTACCAGA-3′
SOX2 transgene rev	5′-TTTGTTTGACAGGAGCGACAAT-3′
LIN28 transgene for	5′-AGCCATATGGTAGCCTCATGTCCGC-3′
LIN28 transgene rev	5′-TAGCGTAAAAGGAGCAACATAG-3′

3.3. Sequencing

Sanger sequencing of a 300 base pair region around the CAG repeat region of the *ATXN2* gene was carried out in an ABI PRISM 310 Genetic Analyzer using the primers SCA2 seq2 forward 5′-CTTGGTCTCGGCGG GC-3′ and SCA2 seq2 reverse 5′-GAGGAGACCGAGGACGAGG-3′.

3.4. RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

RNA was extracted using RNeasy Mini (QIAGEN) and cDNA synthesis was performed using Revert Aid First Strand cDNA synthesis kit (Thermo Scientific) according to the manufacturers' protocols. qRT-PCR was carried out using LightCycler 480 SYBR Green I Master (Roche). Data was plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$. The following primers were used:

OCT4 for	5′-CCCCAGGGCCCCATTTTGGTACC-3′
OCT4 rev	5′-ACCTCAGTTTGAATGCATGGGAGAGC-3′
SOX2 for	5′-TTCACATGTCCCAGCACTACCAGA-3′
SOX2 rev	5′-TCACATGTGTGAGAGGGGCAGTGTGC-3′
NANOG for	5′-AAAGAATCTTCACCTATGCC-3′
NANOG rev	5′-GAAGGAAGAGGAGAGACAGT-3′
REX1 for	5′-TTTCTGAGTACGTGCCCAGGCAA-3′
REX1 rev	5′-CTCTGAGAAAGCATCTCTCCTTC-3′
LIN28 for	5′-AGCCATATGGTAGCCTCATGTCCGC-3′
LIN28 rev	5′-TCAATTCTGTGCCTCCGGGAGCAGGGTAGG-3′
ACTB for	5′-TCAAGATCATTGCTCCTCCTGAG-3′
ACTB rev	5′-ACATCTGCTGGAAGGTGGACA-3′
RPL13A for	5′-TTCCAAGCGGCTGCCGAAGA-3′
RPL13A rev	5′-TTCCGGCCCAGCAGTACCTGT-3′
HSP90AB1 for	5′-TCCGGCGCAGTGTTGGGAC-3′
HSP90AB1 rev	5′-TCCATGGTGCACTTCCTCAGGC-3′

(continued)

GUSB for	5′-TCCGCGCCGACTTCTCTGACA-3′
GUSB rev	5′-AAATGCCGCAGACGCCAGTCC-3′

3.5. Immunocytochemistry

Immunocytochemistry was performed as previously described (Marthaler et al., 2013). The following primary antibodies were used: Anti-OCT4 (Santa Cruz, sc 8628); anti-NANOG (Peprotech, 500P236); anti-TRA1-60 (BioLegend, 330602); anti-SSEA4 (BioLegend, 330402); anti-TUBB3 (Millipore, MAB1637); anti-SMA (Dako, M0851), and anti-SOX17 (Santa Cruz, sc17355); all 1:500. Secondary antibodies used were: Alexa Fluor 594 rabbit anti-goat (A21223) and goat anti-rabbit (A11072), Alexa Fluor 488 donkey anti-rabbit (A21206), donkey anti-goat (A11055), and goat anti-mouse (A11017), all 1:2000, (all Invitrogen).

3.6. Embryoid body differentiation

iPSCs growing in E8 medium (Gibco) on matrigel (Corning Bioscience) were dissociated with EDTA (Gibco) and allowed to form aggregates in none-coated cell culture dishes. On day 3, aggregates were transferred to matrigel-coated dishes and medium was switched to differentiation medium: DMEM/F12 containing 20% FBS, L-glutamine, and non-essential amino acids (all Gibco) for meso- and endoderm induction, or DMEM/F12 containing 50% neurobasal medium, B27, N2, and L-glutamine (all Gibco) for ectoderm induction. Cells were fixed for immunocytochemistry on day 14.

3.7. Verification and authentication

An intact genome with no detectable abnormalities was demonstrated by karyotyping using G-banding (Fig. 1F). Analysis was performed by Cell Guidance Systems, Cambridge, UK.

3.8. Ethical approval

The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (RH), (protocol number H-4-2011-157), Copenhagen, Denmark, and written informed consent was obtained in all cases.

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Fig. 1. H271 c1 is a *bona fide* iPSC line with a normal karyotype. (A) The absence of reprogramming plasmids was confirmed by quantitative PCR on genomic DNA of H271 c1. Fibroblasts 20 days post electroporation or non-electroporated fibroblasts served as positive and negative controls, respectively. Data was normalized to *GAPDH* and plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$. Due to location on the same plasmid, *shp53*, *KLF4*, and *L-Myc* are represented by *OCT4*, *SOX2*, and *LIN28*, respectively. (B) Sequencing of the expanded and the normal *ATXN2* allele showed 44 and 22 trinucleotide repeats, respectively, highlighted in blue. (C) Expression levels of pluripotency genes measured by qRT-PCR. Data were plotted relative to H1 human ESCs. Fibroblasts served as a negative control. Error bars indicate standard error of calculations based on ΔΔCt-values obtained from four housekeeping genes, *ACTB*, *RPL37a*, *HSP90AB1*, and *GUSB*. (D) OCT4, NANOG, TRA1-60, and SSEA4 immunofluorescence images of H271 c1, counterstained with Hoechst. Scale bars, 100 μm. (E) Immunocytochemistry for marker proteins representative of the three germ layers, TUBB3 (ectoderm), SMA (mesoderm), and SOX17 (endoderm), after *in vitro* differentiation of H271 c1 by embryoid body formation. Scale bars, 100 μm. (F) Chromosome analysis of H271 c1 showing a normal 46, XY karyotype.