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Lab Resource: Multiple Cell Lines

Generation of 3 spinocerebellar ataxia type 1 (SCA1) patient-derived induced pluripotent stem cell lines LUMCi002-A, B, and C and 2 unaffected sibling control induced pluripotent stem cell lines LUMCi003-A and B

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ABSTRACT

Spinocerebellar ataxia type 1 (SCA1) is a hereditary neurodegenerative disease caused by a CAG repeat expansion in exon 8 of the *ATXN1* gene. We generated induced pluripotent stem cells (hiPSCs) from a SCA1 patient and his non-affected sister by using non-integrating Sendai Viruses (SeV). The resulting hiPSCs are SeVfree, express pluripotency markers, display a normal karyotype, retain the mutation (length of the CAG repeat expansion in the *ATXN1* gene) and are able to differentiate into the three germ layers *in vitro*.

Resource table.

Unique stem cell lines identifier	LUMCi002-A LUMCi002-B LUMCi002-C LUMCi003-A LUMCi003-B
Alternative names of stem cell lines	LUMCi002-A: LUMC0113iATAX06 and 113-6 LUMCi002-B: LUMC0113iATAX07 and 113-7 LUMCi002-C: LUMC0113iATAX08 and 113-8 LUMCi003-A: LUMC0114iCTRL01 and 114-1 LUMCi003-B: LUMC0114iCTRL02 and 114-2
Institution	Leiden University Medical Center (LUMC), Leiden, The Netherlands
Contact information of distributor	Dr. Ronald A.M. Buijsen (R.A.M.Buijsen@lumc.nl)
Type of cell lines	hiPSC
Origin	Human
Cell source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrating Sendai virus
Multiline rationale	Control (2 clones) and disease (3 clones) pair
Gene modification	No
Type of modification	N/A
Associated disease	Spinocerebellar ataxia type 1 (SCA1)
Gene/locus	*ATXN1*/6p22.3
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	November 30, 2017
Cell line repository/bank	N/A
Ethical approval	The study was approved by the LUMC medical ethics committee (NL45478.058.13/P13.080) and informed consent was obtained from both SCA1 patient and his non-affected sister.

Resource utility

These newly generated hiPSCs are useful to study SCA1 disease mechanisms and therapeutic intervention strategies.

Resource details

Spinocerebellar ataxia type 1 (SCA1) is a rare, autosomal dominant, neurodegenerative disease clinically characterized by progressive ataxia, dysphagia, oculomotor disturbance, pyramidal and extrapyramidal symptoms, sensory deficits as well as mild cognitive decline (Sasaki

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Table 1
Summary of lines.

hiPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of *ATXN1* locus (#CAG repeats)	Disease
LUMCi002-A	113-6	Male	43	Caucasian	29/46	SCA1
LUMCi002-B	113-7	Male	43	Caucasian	29/46	SCA1
LUMCi002-C	113-8	Male	43	Caucasian	29/47	SCA1
LUMCi003-A	114-1	Female	49	Caucasian	29/30	Non-affected control
LUMCi003-B	114-2	Female	49	Caucasian	29/30	Non-affected control

et al., 1996). SCA1 is caused by a CAG triplet repeat expansion in exon 8 of the *ATXN1* gene located on chromosome 6p22-p23 (Orr et al., 1993). In this study, dermal fibroblasts were obtained from a 43 year old man diagnosed with SCA1 and his 49 year old non-affected sister. Fibroblasts were successfully reprogrammed into hiPSCs by using a replication-defective and persistent Sendai virus (SeV) vector installed with *OCT4*, *SOX2*, *KLF4*, and *c-MYC* (Nishimura et al., 2011). The three patient-derived hiPSC clones derived from SCA1 fibroblast line SCA1 2A were named LUMCi002-A, LUMCi002-B, and LUMCi002-C. The two clones derived from control fibroblast line SCA1 2B of the non-affected sister were named LUMCi003-A and LUMCi003-B (Table 1). All hiPSCs showed a typical ES cell like morphology with small and tightly packed cells, a high nucleus to cytoplasm ratio, and well defined nucleoli (Fig. 1A). Furthermore, all hiPSCs were SeVfree at passage 5 (data not shown) and stained positive for the pluripotency markers Oct3/4, Nanog, and SSEA-4 (Fig. 1B). Accordingly, the expression of the pluripotency genes *OCT4*, *NANOG*, and *SOX2* was upregulated in hiPSCs compared to fibroblasts (Fig. 1C). A routine Global Screening Array showed (GSA) no major copy number variations or allelic changes between the original fibroblast cell line and related hiPSC cell lines (Fig. 1D). The CAG repeat size was confirmed by PCR (Fig. 1E) and fragment analysis (data not shown) in these newly established hiPSC lines. Furthermore, all generated hiPSC lines were able to differentiate into all three germ layers *in vitro* as confirmed by immunofluorescent staining for the endodermal marker α-fetoprotein (AFP), the mesodermal marker PECAM-1 (CD31), and the ectodermal marker β3-tubulin (TUBB3) (Fig. 1F). The presence of mycoplasma was tested regularly and all cell lines were negative (Supplementary Fig. S1). All data is present in Table 2.

Materials and methods

Ethical statement

This study was approved by the LUMC scientific ethical committee and informed consent was obtained from both SCA1 patient and his non-affected sister (NL45478.058.13/P13.080).

Generation of hiPSCs

A skin biopsy was obtained from a 43 year old male SCA1 patient and his 43 year old sister. After dissection fibroblast were cultured in medium containing minimum essential medium supplemented with 15% FBS, 2 mM GlutaMAX and 1% penicillin-streptomycin (all ThermoFisher) at 37 °C and 5% CO_2, expanded to passage three and frozen for future use. For reprogramming 1×10^5 fibroblasts were infected with 7.5 MOI SeVdp(KOSM)302 L and seeded on irradiated mouse embryonic fibroblasts (MEFs) in fibroblast media 24 h after transduction. Starting the next day, cells were cultured in DMEM/F12 Glutamax medium with 20% KnockOut Serum Replacement (KOSR), Non-Essential Amino Acids (NEAA), 2-mercapthoethanol, Pen/Strep (all Gibco) and 10 ng/ml bFGF (Peprotech) until hiPSC colonies emerged about 3 weeks later. hiPSC colonies were picked manually and expanded on Vitronectin XF in TESR-E8 media according to manufacturer's instructions (STEMCELL Technologies) (Table 1).

Spontaneous in vitro differentiation of hiPSCs

Aggregates of undifferentiated hiPSCs were harvested using Gentle Cell Dissociation Reagent STEMCELL Technologies) and plated on Vitronectin XF coated glass coverslips in TESR-E8 media according to manufacturer's instructions (STEMCELL Technologies). Cells were cultured in DMEM/F12 with 20% FBS for three weeks with media changes every other day.

Immunofluorescent staining

hiPSCs were fixed in 2% paraformaldehyde for 30 min, RT, permeabilized with 0.1% Triton X-100, blocked in 4% normal swine serum (NSS, DAKO) for 1 h at RT and incubated with primary antibodies in 4% NSS o/n at 4 °C followed by incubation with secondary fluorescent dye-labelled antibodies for 1 h at RT. DAPI was used as nuclear counter staining. Antibodies are listed in Table 2. Images were made on a Leica TCS SP8.

RNA isolation and RT-qPCR

RNA isolation was performed using the ReliaPrep™ Miniprep System (Promega) according to the manufacturer's instructions. 500 ng RNA/reaction was reverse transcribed using the transcriptor first strand cDNA synthesis kit (Roche). qRT-PCR reactions were run on a LightCycler® 480 Real-Time PCR System (Roche) with SensiMix SYBR Hi-ROX Kit (Bioline). Cycle parameters were an initial denaturation of 10 min at 95 °C, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s and extension at 72 °C for 20 s. CT-values were normalized to GAPDH using the ΔΔCT-method. Primer sequences are listed in Table 3.

DNA isolation

Genomic DNA isolation was performed using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions.

Repeat length PCR

The CAG repeat in exon 8 of the *ATXN1* gene was amplified to determine the genotype of the hiPSCs. Cycling conditions were an initial denaturation of 4 min at 95 °C, followed by 35 cycles of 30 s 95 °C, 30 s 60 °C and 1 min 72 °C. The primers are listed in Table 2.

Fragment length analysis

Fragment length analysis was carried out with OneTaq Master Mix (New England Biolabs) on a ABI genetic analyser (ThermoFisher). Cycling conditions were an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 30 s 94 °C, 1 min 60 °C and 2 min 68 °C. The primers are listed in Table 2.

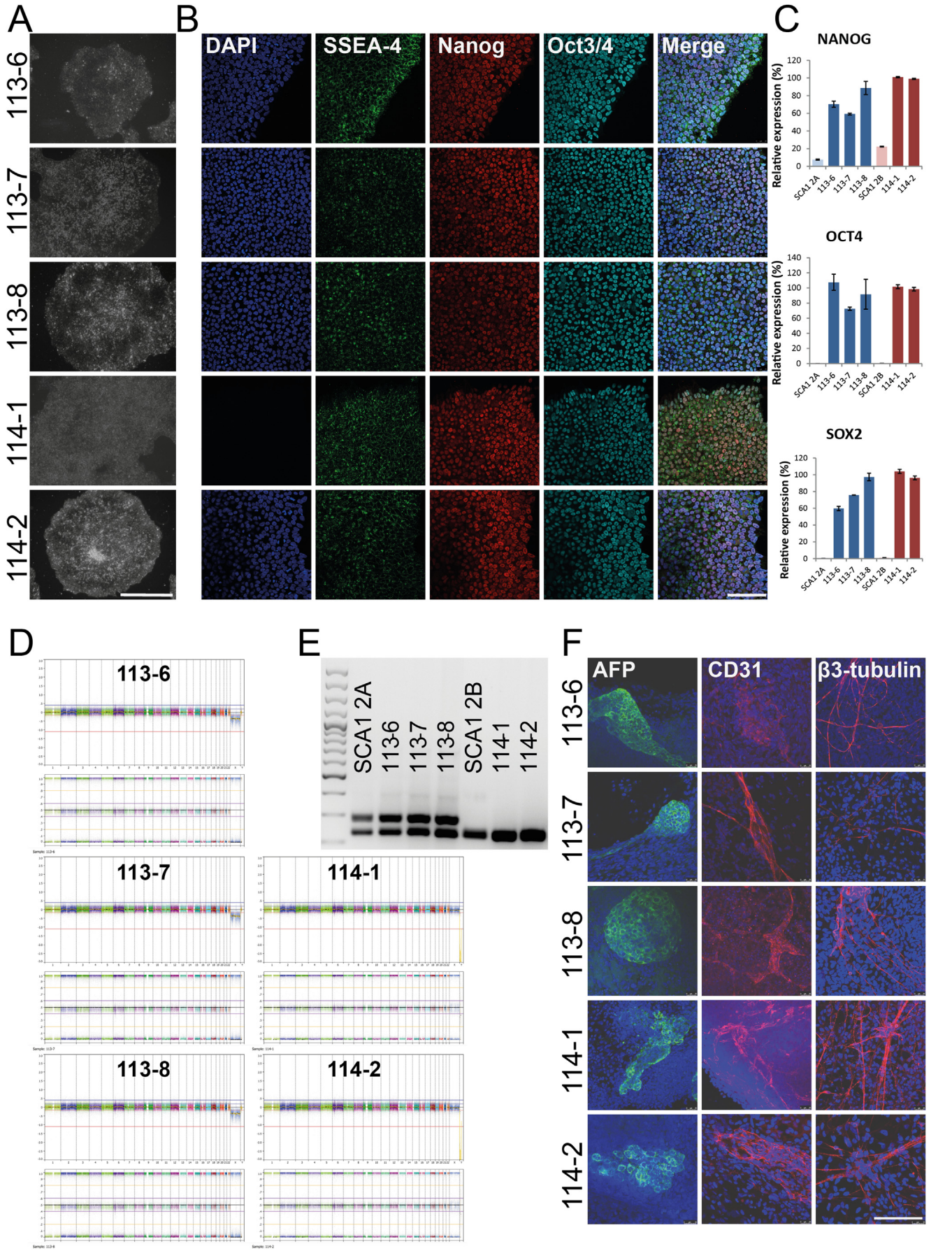

Fig. 1. Characterization of SCA1 hiPSCs LUMCi002-A, B, and C and unaffected sibling control hiPSCs LUMCi003-A and B.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast microscopy	Normal Scale bar represents 1000 μm	Fig. 1 panel A
Phenotype	Qualitative analysis of immunofluorescent staining	Positive staining of pluripotency markers: Oct-3/4, Nanog, SSEA-4 Scale bar represents 100 μm	Fig. 1 panel B
	Quantitative analysis by RT-qPCR	Expression of pluripotency markers: *OCT4*, *NANOG*, *SOX2*	Fig. 1 panel C
Genotype	GSAMD24 v1 Illumina Infinium Snp array 700 k	CNV report resolution 150 kb: No major copy number variations or allelic changes LUMCi002-A, B, and C: 46 XY LUMCi003-A and B: 46 XX	Fig. 1 panel D
Identity	GSAMD24 v1 Illumina Infinium Snp array 700 k	DNA Profiling: Performed Fibroblasts and derived hiPSCs have >99% identical SNPs	Data not shown but available from author Submitted in archive with journal
Mutation analysis	Repeat length PCR and fragment analysis	Fibroblasts and derived hiPSCs have similar CAG repeat lengths	Fig. 1 panel E (PCR) and data not shown but available from author (fragment analysis)
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence: Negative	Supplementary Fig. S1
Spontaneous Differentiation	Qualitative analysis of Immunofluorescent staining	Positive staining of germ layer markers: AFP (endoderm), CD31 (mesoderm), β3-tubulin (ectoderm) Scale bar represents 100 μm	Fig. 1 panel F
Donor screening	N/A		
Genotype additional info	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

Table 3
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	mouse IgG2b anti-Oct-3/4	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
	mouse IgG1 anti-Nanog	1:150	Santa Cruz Biotechnology Cat# sc-293121, RRID: AB_2665475
	mouse IgG3 anti-SSEA-4	1:30	BioLegend Cat# 330402, RRID:AB_1089208
Differentiation markers	mouse IgG2a anti-β3-tubulin	1:4000	Covance Research Products Inc. Cat# MMS-435P, RRID: AB_2313773
	mouse anti-CD31	1:100	Dako Cat# M0823, RRID:AB_2114471
	rabbit IgG anti α-fetoprotein (AFP)	1:25	Quartett, Cat# 2011200530, RRID:AB_2716839
Secondary antibodies	Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:250	Thermo Fisher Scientific Cat# A-21242, RRID:AB_2535811
	Goat anti-Mouse IgG3 Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:250	Thermo Fisher Scientific Cat# A-21151, RRID:AB_2535784
	Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:250	Thermo Fisher Scientific Cat# A-21124, RRID:AB_2535766
	Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:500	Thermo Fisher Scientific Cat# A-11031, RRID:AB_144696
	Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21206, RRID:AB_2535792

Primer

	Target	Forward/Reverse primer (5′–3′)
SeV based vectors (qPCR)	*SeV*	GCAGCTCTAACGTTGTCAAA/CCTGGAGCAAATTCACCATGA
	GAPDH	TCCTCTGACTTCAACAGCGA/GGGTCTTACTCCTTGGAGGC
Pluripotency Markers (qPCR)	*NANOG*	CAGTCTGGACACTGGCTGAA/CTCGCTGATTAGGCTCCAAC
	OCT4	TGTACTCCTCGGTCCCTTTC/TCCAGGTTTTCTTTCCTAGC
	SOX2	GCTAGTCTCCAAGCGACGAA/GCAAGAAGCCTCTCCTTGAA
House-Keeping Genes (qPCR)	*GAPDH*	AGCCACATCGCTCAGACACC/GTACTCAGCGGCCAGCATCG
Genotyping by Repeat length PCR	*ATXN1*	TGGAGGCCTATTCCACTCTG/TGGACGTACTGGTTCTGCTG
Targeted mutation analysis (Fragment length analysis)	*ATXN1*	CCCCAACCGCCAACCCC/GTGGGATCATCGTCTGGTGGG

Mycoplasma detection

The presence of mycoplasma was tested using the MycoAlert™ Mycoplasma Detection Kit (Lonza) according to the manufacturer's instructions.

SNP array

The Global Screening Array (Illumina) was used according to standard protocols, followed by a standard analysis in GenomeStudio software (Illumina) with the GSA manifest files. GenomeStudio Finalreports were used to analyze and visualize in Nexus Discovery (BioDiscovery El Segundo).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.03.018.

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