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Generation of human induced pluripotent stem cell lines (LUMCi051-A,B and LUMCi052-A,B,C) of two patients with Spinocerebellar ataxia type 7

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A B S T R A C T

Spinocerebellar Ataxia Type 7 (SCA7) is an autosomal dominantly inherited disorder, primarily characterized by cerebellar ataxia and visual loss. SCA7 is caused by a CAG repeat expansion in exon 3 of the *ATXN7* gene. We generated human induced pluripotent stem cells (hiPSCs) from peripheral blood-derived erythroblasts from two SCA7 patients (LUMCi051-A,B and LUMCi052-A,B,C) using integration-free episomal vectors. All hiPSC clones express pluripotency factors, show a normal karyotype, and can differentiate into the three germ layers. These lines can be used for *in vitro* disease modeling and therapy testing.

1. Resource table

Unique stem cell lines identifier	LUMCi051-A LUMCi051-B LUMCi052-A LUMCi052-B LUMCi052-C
Alternative name(s) of stem cell lines	LUMCi051-A: Lu051i#1 LUMCi051-B: Lu051i#2 LUMCi052-A: Lu052i#1 LUMCi052-B: Lu052i#2 LUMCi052-C: Lu052i#3
Institution	Leiden University Medical Center (LUMC), Leiden, The Netherlands
Contact information of distributor	Prof. Willeke M.C. van Roon-Mom, W.M.C.van_Roon@lumc.nl
Type of cell lines	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*LUMCi051-A,B:* *Age at biopsy: 26* *Sex: female* *Ethnicity if known: African* *LUMCi052-A,B,C:* *Age at biopsy: 20* *Sex: female* *Ethnicity if known: Caucasian*
Cell Source	Peripheral blood derived $CD71^+$ erythroblasts

(*continued on next column*)

(*continued*)

Unique stem cell lines identifier	LUMCi051-A LUMCi051-B LUMCi052-A LUMCi052-B LUMCi052-C
Clonality	*Clonal*
Method of reprogramming	Episomal vectors (pCXLE-hOCT3/4-shp53-F, pCXLE-hSK, pCXLE-hUL)
Genetic Modification	*Yes*
Type of Genetic Modification	*Hereditary*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*PCR*
Associated disease	*Spinocerebellar ataxia type 7 (SCA7)*
Gene/locus	*ATXN7/Chromosome 3p14.1*
Date archived/stock date	*March 2024*
Cell line repository/bank	Human Pluripotent Stem Cell Registry (hPSCreg)https://hpscreg.eu/cell-line/LUMCi051-A https://hpscreg.eu/cell-line/LUMCi051-B https://hpscreg.eu/cell-line/LUMCi052-A https://hpscreg.eu/cell-line/LUMCi052-B https://hpscreg.eu/cell-line/LUMCi052-C

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(*continued*)

Unique stem cell lines identifier	LUMCi051-A LUMCi051-B LUMCi052-A LUMCi052-B LUMCi052-C
Ethical approval	*Medical ethical review board of Radboud university medical center (CMO file number: 2022–13717).*

2. Resource utility

hiPSCs (LUMCi051-A,B and LUMCi052-A,B,C) were generated from peripheral blood-derived erythroblasts from two SCA7 patients. These hiPSCs can be used for *in vitro* disease modeling and drug discovery.

3. Resource details

Spinocerebellar ataxia type 7 (SCA7) is a rare autosomal dominantly inherited neurodegenerative disorder. Patients suffer from a wide range of symptoms, primarily cerebellar ataxia and loss of vision, which can start from infancy to adulthood. SCA7 is a rare disorder as the prevalence is less than 1:300,000 in the general population. SCA7 is caused by a CAG triplet repeat expansion in exon 3 of the *ATXN7* gene (>34 CAG repeats) located on chromosome 3p14.1. This results in a prolonged polyglutamine tract (PolyQ) in the ATXN7 protein, causing both gain- and loss-of-function, and the formation of protein aggregates. There is an inverse correlation between the age of disease onset and the length of the repeat expansion (Goswami et al., 2022; La Spada, 1993).

We successfully reprogrammed peripheral blood-derived erythroblasts from two SCA7 patients into human induced pluripotent stem cells (hiPSCs) using integration-free episomal vectors encoding pluripotency factors. From one patient two hiPSC clones were further expanded and characterized (LUMCi051-A,B) and from the second patient three hiPSC clones (LUMCi052-A,B,C) (Table 1). All hiPSC lines showed typical iPSC morphology including compact colonies and a high nucleus to cytoplasm ratio (Fig. 1A). In all lines, the vast majority of cells expressed pluripotency markers OCT3/4, NANOG and SSEA-4 (Fig. 1B). In addition, the gene expression of pluripotency genes SOX2, NANOG, and OCT4 was highly upregulated compared to the erythroblasts (Fig. 1C). The trilineage potential of the hiPSCs was determined by random differentiation *in vitro* for three weeks. All lines showed positivity for the trilineage markers TUBB3 (ectoderm), α-SMA (mesoderm) and SOX17 (endoderm) (Fig. 1D). Next, the CAG repeat size in exon 3 of the *ATXN7* gene was confirmed using MiSeq analysis. All hiPSC lines carried one wild type CAG repeat allele and one allele with an expanded CAG repeat in the *ATXN7* gene (Fig. 1E). Other genes involved in polyQ SCA disorders did not show an expanded CAG repeat as determined by a fragment length analysis (Supp. Fig. 1A). Furthermore, no major allelic changes in any of the hiPSC clones were detected using a Global Screening Array (GSA) using a report resolution of ~50 kb (Fig. 1F). All lines were identical to the patient-derived erythroblasts as shown by comparison of the allelic calls (Supp. Fig. 1B). All hiPSC lines were negative in regular tests for mycoplasma (Supp. Fig. 1C). At last, assessment of episome disappearance showed that all hiPSC lines were negative as determined by PCR (Supp. Fig. 1D).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	*Bright field microscopy*	*Normal*	Fig. 1*A*
Phenotype	*Immunocytochemistry*	*Positivity for pluripotency markers NANOG, OCT3/4, SSEA-4.*	Fig. 1*B*
	RT-qPCR	*High gene expression of SOX2, NANOG, OCT4.*	Fig. 1*C*
Genotype	GSAMD24 v3 Illumina Infinium SNP array 760 k	*CNV report resolution 50 kb: No major copy number variations or allelic imbalances*	Fig. 1*F*
Identity	GSAMD24 v3 Illumina Infinium SNP array 760 k	*Erythroblasts and hiPSCs have > 99,99 % identical genotype*	Supplementary Fig. 1*B*
Mutation analysis (IF APPLICABLE)	Fragment length analysis with MiSeq System	*All hiPSC lines carry one normal and one SCA7-sized CAG repeat in exon 3 of the ATXN7 gene*	Fig. 1*E*
	Fragment length analysis with the ABI genetic analyser	*No expansions of CAG repeats of other PolyQ SCA genes*	Supplementary Fig. 1*A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing by luminescence was negative*	Supplementary Fig. 1*C*
Differentiation potential	*In vitro* spontaneous differentiation	*Positive protein expression of germ layer markers TUBB3 (ectoderm), α-SMA (mesoderm) and SOX17 (endoderm)*	Fig. 1*D*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	*N/A*
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	*N/A*
	HLA tissue typing	*N/A*	*N/A*

4. Materials and methods

4.1. Ethical statement

Informed consent was obtained from both patients. The study was approved by the medical ethical review board of Radboud university medical center (CMO file number: 2022–13717).

4.2. Generation of hiPSCs

hiPSCs were generated from erythroblasts as previously described (Perriot et al., 2022). In short, on day 0 peripheral blood mononuclear cells were thawed and cultured in defined Erythroblast medium. On day 4, the erythroblasts were sorted using CD71 microbeads (Miltenyi). On day 11, the erythroblasts were nucleoporated with the Amaxa Nucleofector™ II system (Lonza, T-016 program) using the human CD34 + Nucleofector kit (Lonza) with 2 μg of the following episomes from Addgene: pCXLE-hOCT3/4-shp53-F (27077); pCXLE-hSK (27078); pCXLE-hUL (27080). After nucleoporation the erythroblasts were transferred to Matrigel-coated culture dishes (both from Corning). From day 14 the medium was gradually switched to ReproTeSR medium (Stemcell technologies). Around day 28 newly generated hiPSC colonies were picked manually and maintained in mTeSR1 medium (STEMCELL Technologies) supplemented with 1x Penicillin-Streptomycin (Gibco) on Matrigel-coated wells (Corning). The hiPSCs were passaged with ReLeSR (STEMCELL Technologies) at 1:15 ratio every 5–7 days and were incubated at 37°C/5% CO_2.

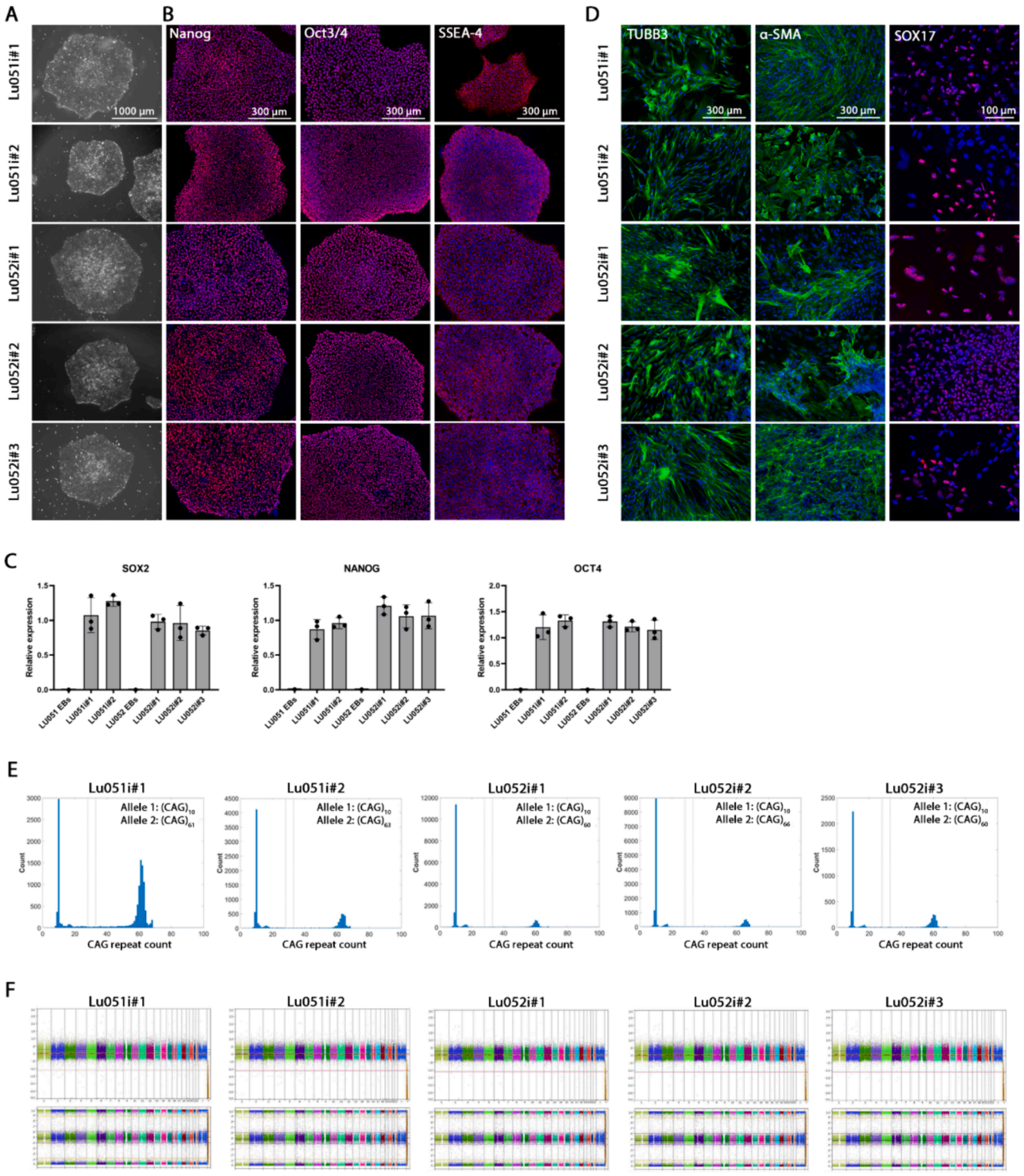

Fig. 1. Characterization of hiPSCs LUMCi051-A, B and LUMCi052-A, B, C.

4.3. Immunofluorescent staining for pluripotency markers

HiPSCs at passage number 12 were fixed in 4 % paraformaldehyde (PFA) for 10 min at room temperature (RT). The cells were incubated with primary antibodies (Table 2) overnight at 4 °C. After washing, the cells were incubated with secondary fluorescent antibodies (Table 2) and DAPI (Sigma Aldrich, D9542) for three hours at RT. Pictures were generated using the Leica DM5500 microscope (Leica Microsystems).

4.4. Trilineage differentiation in vitro and immunofluorescent staining

One day after passaging the hiPSCs (passage number 12), the medium was switched to DMEM/F12 (Thermo Fisher Scientific) supplemented with 20 % FBS. After three weeks of daily medium changes, the cells were fixed in 4 % PFA for 10 min at RT. The cells were incubated with primary antibodies (Table 2) overnight at 4 °C. After washing the cells were incubated with the secondary fluorescent antibodies (Table 2) and DAPI for three hours at RT. Pictures were generated with the Leica DM5500 microscope (Leica Microsystems).

Table 2
Reagents details.

	Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency markers	*Mouse anti-Nanog*	*1:150*	*Santa Cruz, sc-293121*	*AB_2665475*
	Mouse anti-OCT3/4	*1:200*	*Stemcell Technologies, 60,093*	*AB_2801346*
	Mouse IgG3 anti-SSEA-4	*1:30*	*Biolegend, 330,402*	*AB_1089208*
Differentiation markers	*Mouse anti-TUBB3*	*1:4000*	*Biolegend, 801,202*	*AB_2728521*
	Mouse anti- α-SMA	*1:3000*	*Sigma, A2547*	*AB_476701*
	Goat anti-SOX17	*1:100*	*R&D systems, AF1924*	*AB_355060*
Secondary antibodies	*Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594*	*1:500*	*Thermo Fisher Scientific, A11005*	*AB_2534073*
	Goat anti-Mouse IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	*1:500*	*Thermo Fisher Scientific, A11001*	*AB_2534069*
	Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 594	*1:500*	*Thermo Fisher Scientific, A11058*	*AB_2534105*

	Primers		
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Pluripotency markers (RT-qPCR)	*SOX2*	*145 bp*	*GCTAGTCTCCAAGCGACGAA/ GCAAGAAGCCTCTCCTTGAA*
	NANOG	*150 bp*	*CAGTCTGGACACTGGCTGAA/ CTCGCTGATTAGGCTCCAAC*
	OCT4	*150 bp*	*TGTACTCCTCGGTCCCTTTC/ TCCAGGTTTTCTTTCCCTAGC*
House-Keeping Gene (RT-qPCR)	*GAPDH*	*150 bp*	*AGCCACATCGCTCAGACACC/ GTACTCAGCGGCCAGCATCG*
House-Keeping Gene (PCR)	*β-ACTIN*	*249 bp*	*CATGTACGTTGCTATCCAGGC/ CTCCTTAATGTCACGCACGAT*
SCA7 genotype (MiSeq)	*ATXN7*	*+/- 350 bp and 500 bp*	*GATGTGTATAAGAGACAGGGAAAGAATGTCGGAGCGGG/ CGTGTGCTCTTCCGATCTGCCTCAACCCACAGATTCCA*
Episomal fading	*EBNA1*	*308 bp*	*AGATGACCCAGGAGAAGGCCCAAGC/ CAAAGGGGAGACGACTCAATGGTGT*

4.5. RNA isolation and RT-qPCR

For RNA isolations (at passage number 12) the ReliaPrep™ Miniprep System (Promega) was used according to manufacturer's instructions. cDNA of 500 ng RNA was generated using the Transcriptor First Strand cDNA Synthesis Kit (Roche). Gene expression levels were determined by RT-qPCR with the CFX96 system (Bio-Rad) with SensiMix SYBR Hi-ROX Kit (Bioline) and the primers listed in Table 2. The obtained Cq values were normalized to GAPDH.

4.6. Genomic DNA isolation

DNA was isolated with the ReliaPrep™ gDNA Tissue Miniprep System (Promega) according to manufacturer's instructions.

4.7. Episomal fading

The clearance of episomes at passage number 14 was determined on DNA by PCR. The cycling conditions were a denaturation step of 4 min at 95 °C in the beginning, followed by 35 cycles of 30 s 95 °C, 30 s 58 °C and 30 s 72 °C. The primers are listed in Table 2.

4.8. Chromosomal abnormalities and DNA fingerprinting

Copy Number Variation (CNV) profiles were determined at passage number 15 using the Infinium Global Screening Array-24 v3.0 (Illumina Inc.), processed in in GenomeStudio software v2.0 (Illumina Inc), and visually inspected in Biodiscovery Nexus CN10.0 (Biodiscovery Inc.) using methods, thresholds and analysis settings described previously (Brosens et al., 2016). Using the paired analysis module in the Genomestudio V2.0 software, we compared the genotypes derived from the GSV3 array to confirm the identity of patient germline and hiPSCs DNA prior to CNV analysis.

4.9. miSeq assay

The sequence coding for the ATXN7 polyglutamine tract was amplified from genomic DNA using tailed ATXN7 primers (Table 2). PCR products were cleaned-up using Ampure XP DNA beads (Beckman Coulter). A second PCR was performed to extend the amplicons with Illumina adapters and sample-identification barcodes. PCR products containing the barcodes were cleaned-up using Ampure XP DNA beads and the sequencing library was then quality controlled using the Qubit fluorometer (ThermoFisher) and Bioanalyzer Lab-on-a-Chip (Agilent) followed by sequencing on the MiSeq platform (Illumina). The CAG repeat size and composition was determined from the sequencing reads using a custom made analysis pipeline in MATLAB (MathWorks). The analysis script is available upon request.

4.10. Fragment length analysis for PolyQ genes

Fragment length analysis was performed with OneTaq Master Mix (New England Biolabs) on an ABI genetic analyser (ThermoFisher). Primer sequences are available upon request.

4.11. Mycoplasma test

The MycoAlert™ Mycoplasma Detection Kit (Lonza) was used to detect myocoplasma at passage number 10.

CRediT authorship contribution statement

Linde F. Bouwman: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Milou E.M. Joosen:** Writing – review & editing, Data curation. **Ronald A.M. Buijsen:** Writing – review & editing, Software, Methodology, Data curation. **Linda M. van der Graaf:** Writing – review & editing, Methodology, Data curation. **Barry A. Pepers:** Writing –

review & editing, Methodology, Data curation. **Bas J.B. Voesenek:** Writing – review & editing, Software, Data curation. **Erwin Brosens:** Writing – review & editing, Software, Data curation. **Bart P.C. van de Warrenburg:** Writing – review & editing, Funding acquisition, Conceptualization. **Willeke M.C. van Roon-Mom:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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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.2024.103462.

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