



Lab Resource: Multiple Cell Lines

Generation of two induced pluripotent stem cell lines from a 33-year-old central core disease patient with a heterozygous dominant c.14145_14156delCTACTGGGACA (p.Asn4715_Asp4718del) deletion in the RYR1 gene



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A B S T R A C T

Central core disease (CCD) is a congenital disorder that results in hypotonia, delayed motor development, and areas of reduced oxidative activity in the muscle fibre. Two induced pluripotent stem cell (iPSC) lines were generated from the lymphoblastoid cells of a 33-year-old male with CCD, caused by a previously unreported dominant c.14145_14156delCTACTGGGACA (p.Asn4715_Asp4718del) deletion in the *RYR1* gene. Both lines demonstrated typical morphology, pluripotency, tri-lineage differentiation, and had a normal karyotype. As the first published iPSC model of CCD caused by an *RYR1* variant these lines are a potential resource for further investigation of *RYR1*-related myopathies in a human context.

Resource table

Unique stem cell lines identifier	1. HPII005-A 2. HPII005-B
Alternative name(s) of stem cell lines	1. HPII005-A: RYR1-5-9214-iPSC clone R5 2. HPII005-B: RYR1-5-9214-iPSC clone R11
Institution	Harry Perkins Institute of Medical Research
Contact information of distributor	Dr Joshua Clayton: joshua.clayton@perkins.org.au
Type of cell lines	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 33 Sex: Male Ethnicity if known: European
Cell Source	EBV-immortalised lymphoblastoid cell line (LCL)

(continued on next column)

Resource table (continued)

Clonality	Clonal
Method of reprogramming	Sendai virus (CytoTune™ 2.0)
Genetic Modification	Yes
Type of Genetic Modification	Spontaneous variant
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR to confirm clearance of Sendai virus
Associated disease	Central core disease of muscle; CCD (OMIM#117000)
Gene/locus	Ryanodine Receptor 1, Skeletal Muscle (<i>RYR1</i>), NM_000540.3: c.14145_14156delCTACTGGGACAA (p. Asn4715_Asp4718del)

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Resource table (continued)

Date archived/stock date	Archived at Harry Perkins Institute: 27th July 2022
Cell line repository/bank	1. HPIi005-A: https://hpscreg.eu/cell-line/HPIi005-A 2. HPIi005-B: https://hpscreg.eu/cell-line/HPIi005-B
Ethical approval	Patient peripheral lymphocytes were collected with appropriate ethics approvals and consent from the Comité de Protection des Personnes (Est IV DC-2012-1693). The patient's LCLs were banked by Genethon; activity authorization No. AC-2018-3156, import/export authorization No. IE-2018-994. The study was approved by the University of Western Australia's Human Research Ethics Committee (approval number: RA/4/20/1008).

1. Resource utility

These are the first published iPSC lines from a patient with CCD caused by an *RYR1* variant. Since there is no approved treatment for CCD there is an imperative need for clinically relevant models of the disorder. These lines may facilitate disease modelling and development of therapies for *RYR1*-related myopathies.

2. Resource details

RYR1-related myopathies encompass several sub-types of congenital myopathy, including central core disease (CCD). CCD presents in infancy with hypotonia, proximal weakness, motor developmental delay and unique areas with reduced oxidative activity along the longitudinal axis of the muscle fibre (central cores) (Jungbluth et al., 2018). There is currently no cure or approved treatment for *RYR1*-related myopathy (Beaufils et al., 2022). Clinically relevant and tractable models of the disorder that reflect the genetic background of patients may support the development of new treatments.

In this study, we reprogrammed EBV-immortalised lymphoblastoid cells (LCLs) from a 33-year-old patient assigned male at birth, harbouring a heterozygous deletion (c.14145_14156delCTACTGGGACAA, p.Asn4715_Asp4718del) in the *RYR1* gene. This variant has not been previously reported. The patient presented with moderate muscular weakness, not evolving since childhood, Achilles retractions, and late walking acquisition (16 months). The patient was clinically diagnosed with congenital myopathy, and muscle biopsy revealed central cores. Both parents were unaffected. The variant was not screened in the parents. The variant is not present in gnomAD and changes the protein coding length due to an in-frame deletion of four amino acids (NYWD). These residues are completely conserved through to *X. tropicalis*, via MutationTaster2 (Schwarz et al., 2014), with a PhyloP100 score of 9.898. The variant is located in exon 97/106 (ENST00000359596) and affects the transmembrane (pore) domain of the protein (Meissner, 2017).

LCLs obtained from the patient (Genethon) were reprogrammed to iPSCs using the CytoTune™ 2.0 Sendai Reprogramming Kit. Clones were initially selected for characterisation on the basis of their morphology. The two clones characterised here demonstrated a high nucleus to cytoplasm ratio, tightly packed colonies, and limited spontaneous differentiation (Fig. 1A). Both clones demonstrated enrichment of pluripotency markers (*OCT4*, *CRYPTO*, *SOX2* and *NANOG*) by qRT-PCR relative to non-transduced LCLs, at levels comparable to a control iPSC line (HC01-5) (Fig. 1B). Both clones uniformly expressed

pluripotency markers (*OCT4*, *SSEA4*, *TRA-1-60*, and *SOX2*) by immunocytochemistry (Fig. 1C). Following trilineage differentiation, both clones produced a high proportion of cells that stained positively for key mesodermal (Brachyury), ectodermal (OTX2) and endodermal (SOX17) proteins (Supp Fig. 1A). At the transcript level, differentiated cells from both clones demonstrated robust induction of mesodermal (*TBXT*, *BMP4*), ectodermal (*OTX2*, *PAX6*), and endodermal (*SOX17*, *GATA4*) markers relative to undifferentiated iPSCs (Fig. 1D).

Both clones were cleared of EBV by passage 15 (Fig. 1E), and Sendai virus by passage 16 (Fig. 1F). Cultures were also confirmed to be clear of mycoplasma at passage 14 (Supp Fig. 1B). Both clones were screened for eight common karyotypic iPSC abnormalities using the hPSC Genetic Analysis Kit (StemCell Technologies). No statistically significant chromosomal abnormalities were detected in either clone (Supp Fig. 1C). G-banding indicated that both clones possessed a normal male karyotype (46, XY) (Fig. 1G). Sanger sequencing of both clones confirmed that the expected c.14145_14156del CTACTGGGACAA (p.Asn4715_Asp4718del) deletion was present in exon 97 of the *RYR1* gene (Fig. 1H). Both iPSC lines were also confirmed to match the patient identity of parental LCLs at all 22 loci tested by STR typing. All characterisation details are summarised in Table 1.

3. Materials and methods

3.1. Reprogramming and culture

LCLs were cultured in RPMI-1640 medium (Gibco), supplemented with 1% L-glutamine (Gibco) and 10% FBS (Gibco). LCLs were reprogrammed to iPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher). Briefly, 3×10^5 cells were transduced and plated on growth factor-reduced (GFR) Matrigel® (#354230, Corning) diluted 1:100 in DMEM/F12, and gradually transitioned from RPMI to ReproTeSR. Resulting iPSC clones were expanded in mTeSR™ Plus on GFR Matrigel® and were passaged 1:10–1:20 every 3–5 days at 60–80% confluence using Versene (ThermoFisher). Healthy control iPSCs (HC01-5) were gifted from Prof. Rhonda Bassel-Duby (Min et al., 2019). All cells were maintained at 37°C with 5% CO₂ (StemCell Technologies).

3.2. DNA and RNA extraction

Genomic DNA (gDNA) was extracted using the QIAamp® DNA mini kit (Qiagen). RNA was extracted using the RNeasy mini kit (Qiagen). cDNA was synthesised using the SuperScript® III First-Strand Synthesis System for RT-PCR (Thermo Fisher).

3.3. Polymerase chain reaction (PCR) and reverse transcriptase PCR (RT-PCR)

Polymerase Chain Reactions (PCR) contained 2X GoTaq Green Master Mix (Promega) and were performed using a C1000™ Cycler (BioRad). Cycling conditions for EBV and Sendai virus detection were: 95°C/2 min, (95°C/30 s, 60°C/30 s and 72°C/40 s) × 35, 72°C/5 min. Cycling conditions for *RYR1* variant confirmation were 95°C/5 min, (95°C/30 s, 65°C/30 s–0.5°C/cycle, 72°C/30 s) × 15, (95°C/30 s, 57°C/30 s, 72°C/30 s) × 30, 72°C/5 min. Primers are listed in Table 2.

3.4. Quantitative PCR (qRT-PCR)

qRT-PCRs were performed using the Rotor-Gene SYBR Green RT-PCR Kit on a Rotor-Gene-Q System (Qiagen). Cycling conditions: 95°C/5 min, (95°C/10 s, 60°C/15 s) × 45, followed by melt curve analysis. Data were

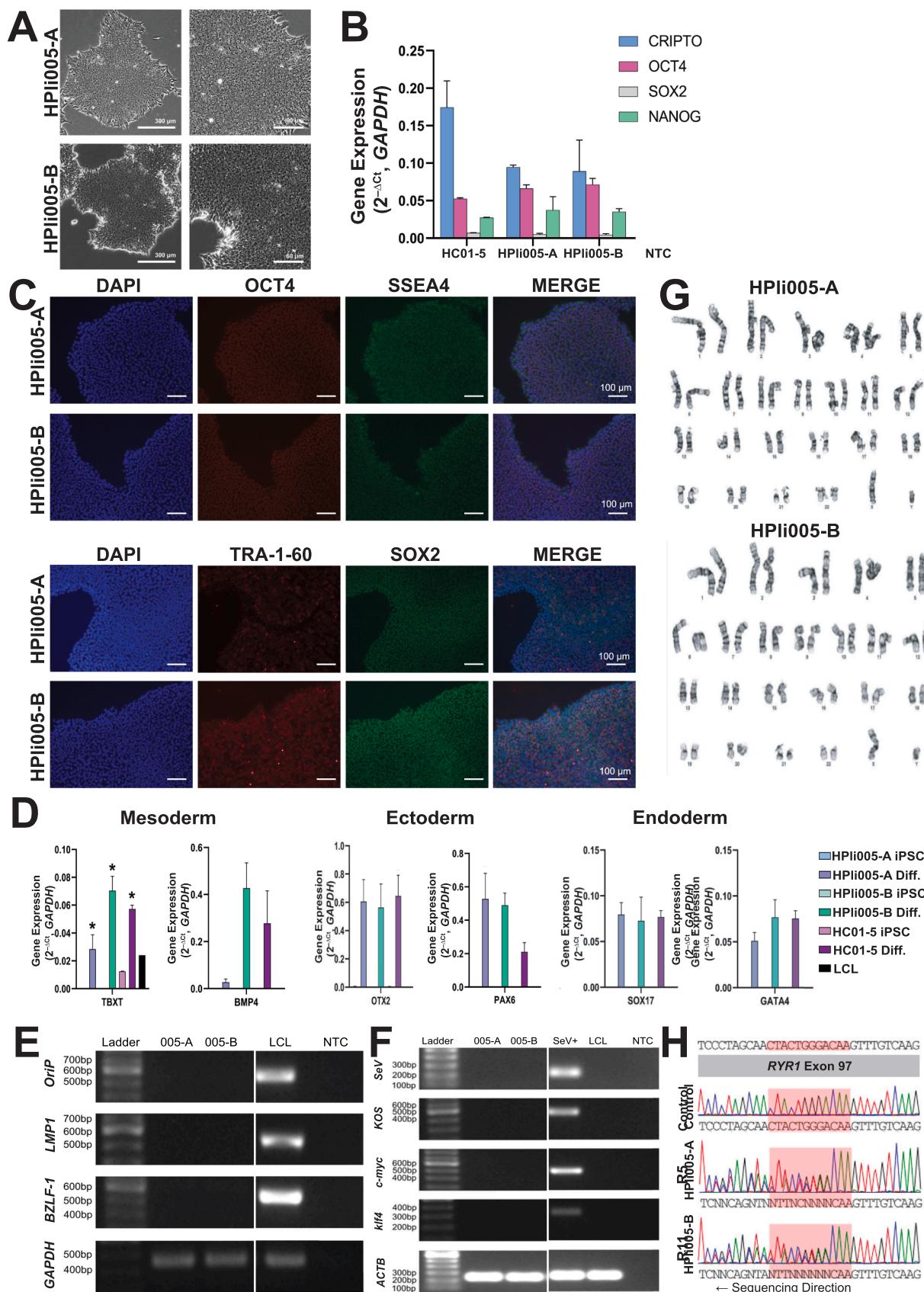


Fig. 1. Cellular and molecular characterisation of HPIi005-A and HPIi005-B iPSC lines.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology phenotype	Photography Bright field Qualitative analysis (immunocytochemistry) Quantitative analysis (qRT-PCR)	At P16: Normal At P16: Positive for OCT4, SOX2, SSEA4, TRA-1–60	Fig. 1A Fig. 1C
Genotype	Karyotype (G-banding) and resolution	At P16: Expression of OCT4, SOX2, NANOG, CRIPTO At P17: 46, XY (normal male karyotype) Banding Resolution: 400 bands per haploid set	Fig. 1B Fig. 1G
Identity Mutation analysis	STR analysis Sequencing Southern Blot OR WGS	15 metaphases counted, 5 analysed At P15: Matched to parental LCLs at 22/22 STR loci At P15: Heterozygous RYR1 deletion; NM_000540.3, c.14145_14156delCTACTGGGACA (p.Asn4715_Asp4718del)	Available with authors Fig. 1H N/A Supplementary Fig. 1B
Microbiology and virology Differentiation potential	Directed differentiation Mycoplasma	Not performed At P14: Negative by PCR	Fig. 1C (qPCR data) Supplementary Fig. 1A (immunocytochemistry)
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	Not performed Not performed	

normalised to *GAPDH*.

3.5. STR typing

STR typing was performed by PathWest Diagnostic Genomics (Perth, WA) using the QSTR Plus assay (Elucigene).

3.6. Sanger sequencing

PCR products were purified using a QIAquick PCR Purification kit (Qiagen). Sanger sequencing was conducted by the Australian Genome Research Facility (AGRF; Perth, WA).

3.7. *Mycoplasma* testing

Mycoplasma clearance was assayed using the ATCC Universal *Mycoplasma* Contamination Kit (ATCC).

3.8. Karyotype analysis

Both clones were screened for eight common chromosomal abnormalities using the hPSC Genetic Analysis Kit (StemCell Technologies) and Bio-Rad CFX qPCR machine. G-banding karyotyping was conducted by PathWest Diagnostic Genomics (Perth, WA, Australia).

3.9. Germ layer differentiation

Trilineage differentiation potential was performed using the STEM-diffTM trilineage differentiation kit (Stem Cell Technologies) at P16. Cells were harvested using TrypLE Express (ThermoFisher), pelleted for RNA extraction, or fixed in 4% paraformaldehyde (10 min, room temperature) for immunocytochemistry.

3.10. Immunocytochemistry

For pluripotency analysis, iPSCs were plated in 96-well Nunc polymer optical bottom plates, then fixed and stained for OCT4, SSEA4, SOX2, or TRA-1–60, according to the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (ThermoFisher). For germ layer analysis, fixed cells were permeabilised with 0.4% TritonTM X-100. Cells were blocked for 1 h at room temperature in blocking buffer (1% TritonTM X-100, 10% donkey serum and 0.1% BSA in PBS). Cells were incubated overnight at 4°C with antibodies specific to OTX2, SOX17, or Brachyury at 10 µg/mL, then incubated for 1 h with a fluorophore-conjugated secondary antibody. Nuclei were stained with NucBlue fixed cell stain (ThermoFisher). Cells were imaged using an Olympus IX71 microscope and CellSens software. Antibody details are listed in Table 2.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rhonda Taylor reports financial support was provided by Stan Perron Charitable Foundation.

Data availability

Data will be made available on request.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	RRID
Pluripotency marker	Rabbit anti-OCT4	1:200	Thermo Fisher Cat#A24867	AB_2650999
	Mouse anti-SSEA4	1:200	Thermo Fisher Cat#A24866	AB_2651001
	Rat anti-SOX2	1:200	Thermo Fisher Cat#A24759	AB_2651000
	Mouse anti-TRA-1-60	1:200	Thermo Fisher Cat#A24868	AB_2651002
Secondary antibody	Alexa Fluor™ 594 donkey anti-rabbit	1:250	ThermoFisher #A21207	AB_141637
	Alexa Fluor™ 488 goat anti-mouse IgG3	1:250	ThermoFisher #A24877	AB_2651008
	Alexa Fluor™ 488 donkey anti-rat	1:250	ThermoFisher #A24876	AB_2651007
	Alexa Fluor™ 594 goat anti-mouse IgM	1:250	ThermoFisher #A21044	AB_2535713
Differentiation marker (ectoderm)	Alexa Fluor™ 488 donkey anti-goat IgG	1:500	ThermoFisher #A-11055	AB_2534102
	Goat Anti-Human Otx2 Polyclonal antibody	1:100 (10 µg/mL)	R&D Systems #AF1979	AB_2157172
Differentiation marker (mesoderm)	Human/Mouse Brachyury Polyclonal antibody	1:100 (10 µg/mL)	R&D Systems #AF2085	AB_2200235
Differentiation market (endoderm)	Goat Anti-Human Sox17 Polyclonal antibody	1:100 (10 µg/mL)	R&D Systems #AF1924	AB_355060
PCR Primers				
	Target	Size of band	Forward/Reverse primer (5'-3')	
Targeted mutation sequencing	<i>RYR1</i> Exon 97	355 bp	F: GTTCAGCCAACCCCTGCGTGG / R: CCACCACGCTTGGCCTTGATG	
	<i>OriP</i>	544 bp	F: TCGGGGGTGTAGAGACAAC / R: TGAAGCAGGCGTGGTTCAA	
	<i>BZLF-1</i>	637 bp	F: CACCTCAACCTGGAGACAAT / R: TGAAGCAGGCGTGGTTCAA	
	<i>LMP-1</i>	617 bp	F: ATGGAACACGACCTTGAGA / R: TGAGCAGGATGAGGTCTAGG	
EBV screening	<i>Ebna1</i>	61 bp	F: ATCAGGGCCAAGACATAGAGA / R: GCCAATGCAACTTGGACGTT	
	<i>GAPDH</i>	452 bp	F: ACCACAGTCCATGCCATCAC / R: TCCACCACCTGTTGCTGTA	
	<i>SeV</i>	181 bp	F: GGATCACTAGGTGATATCGAGC / R: ACCAGACAAGAGTTAACAGAGATAATGTATC	
	<i>KOS</i>	528 bp	F: ATGCACCGCTACGACGTGAGGGC / R: ACCTTGACAATCCTGATGTGG	
SeV screening	<i>Klf-4</i>	410 bp	F: TTCCCTGCATGCCAGAGGAGCCC / R: AATGTATCGAAGGTGCTCAA	
	<i>c-myc</i>	532 bp	F: TAACTGACTAGCAGGCTTGTG / R: TCCACATACAGTCTGGATGATGATG	
	<i>ACTB</i>	234 bp	F: GGACTTCGAGCAAGAGATGG / R: AGCACTGTGTTGGCGTACAG	
qRT-PCR Primers				
	Target	Size of band	Forward/Reverse primer (5'-3')	
Pluripotency markers	<i>CRIPTO</i>	66 bp	F: CGGAACGTGAGCACGATGT / R: GGGCAGCCAGGTGTCATG	
	<i>OCT4</i>	63 bp	F: GGGTTTTGGGATTAAGTCTTCA / R: GCCCCCACCTTGTGTT	
	<i>SOX2</i>	63 bp	F: CAAAAATGGCCATGCAGGTT / R: AGTTGGGATCGAACAAAAGCTATT	
	<i>NANOG</i>	111 bp	F: ACAACTGGCCGAAGAATAGCA / R: GGTTCCAGTCGGTTCAC	
Housekeeper genes	<i>EEF2</i>	142 bp	F: CCTTGTGGAGATCCAGTGTCC / R: CTCGTTGACGGGCAGATAGG	

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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.2023.103258>.

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