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Generation and characterization of human induced pluripotent stem cell lines from a familial Alzheimer's disease PSEN1 A246E patient and a non-demented family member bearing wild-type PSEN1

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Abstract

The induced pluripotent stem cell (iPSC) lines UOWi002-A and UOWi003-A were reprogrammed from dermal fibroblasts via mRNA transfection. Dermal fibroblasts from a 56 year old female caucasian familial Alzheimer's disease patient carrying A246E mutation in the PSEN1 gene (familial AD3, autopsy confirmed Alzheimer's disease) and a 75 year old female non-demented control from the same family bearing the wild-type PSEN1 A246 genotype were obtained from the Coriell Institute (AG06848 and AG06846, respectively). The generated iPSCs were characterized and pluripotency was confirmed. The PSEN1 genotype was maintained in both iPSC lines.

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Lab resource: Multiple Cell Lines

Generation and characterization of human induced pluripotent stem cell lines from a familial Alzheimer's disease PSEN1 A246E patient and a non-demented family member bearing wild-type PSEN1

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A B S T R A C T

The induced pluripotent stem cell (iPSC) lines UOWi002-A and UOWi003-A were reprogrammed from dermal fibroblasts via mRNA transfection. Dermal fibroblasts from a 56 year old female caucasian familial Alzheimer's disease patient carrying A246E mutation in the *PSEN1* gene (familial AD3, autopsy confirmed Alzheimer's disease) and a 75 year old female non-demented control from the same family bearing the wild-type *PSEN1* A246 genotype were obtained from the Coriell Institute (AG06848 and AG06846, respectively). The generated iPSCs were characterized and pluripotency was confirmed. The *PSEN1* genotype was maintained in both iPSC lines.

Resource table.

Unique stem cell lines identifier	UOWi002-A UOWi003-A
Alternative names of stem cell lines	iPSC 6846.2 (UOWi002-A) iPSC 6848.2 (UOWi003-A)
Institution	Illawarra Health and Medical Research Institute, University of Wollongong
Contact information of distributor	Lezanne Ooi, lezanne@uow.edu.au
Type of cell lines	iPSC
Origin	Human
Cell source	Dermal fibroblast
Clonal	Single clone
Method of reprogramming	Transgene free
Multiline rationale	Family members, one demented patient with a familial Alzheimer's disease mutation and one non-demented control
Gene modification	No
Type of modification	N/A
Associated disease	Non-demented control (UOWi002-A) Familial Alzheimer's disease (UOWi003-A)
Gene/locus	N/A (UOWi002-A) *PSEN1* A246E (UOWi003-A)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	24/04/2018
Cell line repository/bank	N/A
Ethical approval	HE13/299 University of Wollongong Human Research Ethics Committee

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UOWi002-A	UOWi002-A	Female	75	Caucasian, Canadian	*PSEN1* A246	Non-demented control
UOWi003-A	UOWi003-A	Female	56	Caucasian, Canadian	*PSEN1* A246E	Familial Alzheimer's disease

Resource utility

These iPSC lines can be used as a familial Alzheimer's disease cell line model and an escapee non-demented control from the same family.

Resource details

Dermal fibroblasts from a 56 year old female Alzheimer's disease patient and a 75 year old female escapee from the same family (Nee et al., 1983) were obtained from the Coriell Institute (AG06848 and AG06846, respectively, Table 1). The StemMACS mRNA Reprogramming kit (Miltenyi Biotec) was used to generate the iPSC lines (UOWi003-A and UOWi002-A), following the manufacturer's instructions. The iPSC colonies were isolated and expanded as individual clones. The A246E mutation in the *PSEN1* gene was confirmed present in the UOWi003-A cell line and absent in the UOWi002-A cell line (Fig. 1A), consistent with the parental fibroblast lines. The selected clone for each cell line showed a normal karyotype with no abnormality detected in 15 cells at 400 bands per haploid set for either of the iPSC lines (Fig. 1B), and showed normal iPSC morphology (Fig. 1C). The iPSC lines were authenticated against their respective fibroblast lines via short tandem repeat (STR) profiling. Characterization of the clones was carried out by immunostaining, showing expression of the pluripotent markers Oct4 and SSEA-4 (Fig. 1C) and quantitative RT-PCR (qRT-PCR) showed increased mRNA expression of the pluripotent genes *POU5F1* by > 4000 fold and *NANOG* by 100 fold (Fig. 1D) in the iPSCs in comparison to their parental fibroblasts. Pluripotency was confirmed via PluriTest (Müller et al., 2011), with the UOWi002-A and UOWi003-A lines obtaining a Pluripotency Score of 24.16 and 18.64, respectively and a Novelty Score of 1.46 and 1.47, respectively (Fig. 1E). Differentiation potential of UOWi002-A and UOWi003-A was confirmed by differentiation into endodermal, mesodermal or ectodermal lineages. cDNA from each lineage was pooled in a 1:1:1 ratio and analysed using TaqMan hPSC Scorecard. Results showed upregulation of specific genes of the endoderm, mesoderm and ectoderm layer, while pluripotent genes were downregulated (Fig. 1F). (See Table 2.)

Materials and methods

Reprogramming of dermal fibroblasts into iPSCs

Fibroblasts were plated on Matrigel-coated plates (Corning) and cultured in Stemgent Pluriton Reprogramming Medium before reprogramming was carried out with the StemMACS mRNA Reprogramming kit (Miltenyi Biotec, #130-104-460). After daily mRNA transfection for 11 days, the iPSC colonies that appeared in the cultures were manually transferred to another Matrigel-coated plate for isolation and expansion, and maintained in TeSR-E8 (Stemcell Technologies) at 37 °C and 5% CO_2. This study was approved by the University of Wollongong Human Research Ethics Committee (HE13/299).

Sequencing

DNA was extracted using the ISOLATE II Genomic DNA Kit (Bioline) and amplified by PCR using MyTaq HS DNA Polymerase (Bioline) with *PSEN1* primers (Table 3). Sequencing reactions were performed using ABI BigDye Terminator v3.1 Ready Reaction Mix, separated using 3500xL Genetic Analyzer (Applied Biosystems) and analysed using BioEdit (Fig. 1A).

Karyotyping

Karyotyping of the iPSCs was performed by Sullivan Nicolaides Pathology Pty Ltd. (Bowen Hills, Australia) (Fig. 1B).

Immunofluorescence staining

iPSC colonies were fixed with 4% paraformaldehyde for 10 min and permeabilized with 0.05% Triton-X for 15 min. Blocking was performed by incubation with 5% goat serum for 1 h at 22 °C and primary antibodies (Table 3) were incubated overnight at 4 °C. Samples were incubated with secondary antibody (Table 3) for 1 h at 22 °C followed by RedDot2 (1:200, Biotium) or Hoescht 33,342 (1 μg/ml, Life Technologies) for 10 min. Images were captured on a confocal microscope (Leica DMI6000B) and acquired using LAS AF software (Leica Microsystems) (Fig. 1C).

Quantitative reverse transcription polymerase chain reaction

mRNA was extracted using TriReagent (MRC Gene), DNA was removed using Turbo DNAse kit (ThermoFisher Scientific) and cDNA was generated using Tetro Reverse Transcriptase (Bioline) as per manufacturer's instructions. *POU5F1* and *NANOG* expression was assessed in triplicate using SensiFast SYBR (Bioline) on Corbett RotorGene3000 (ThermoFisher Scientific), using *GAPDH* and *HPRT1* housekeeper genes (Fig. 1D, Table 3). The efficiency of each sample was determined using LinRegPCR (Ruijter et al., 2009) and analysis was performed using the ΔΔCt method.

PluriTest

mRNA was analysed using Illumina HT 12v4 gene expression microarray via PluriTest (Müller et al., 2011) (Fig. 1E).

TaqMan hPSC scorecard

Direct differentiation of iPSC colonies to the three germ layers was performed. Endoderm was induced with STEMdiff Definitive Endoderm Kit (Stemcell Technologies). Mesoderm was induced using Mesoderm base medium (DMEM/F12, 0.5% FBS, 1% GlutaMAX, 1% MEM Non-essential aminoacids, 55 μM 2-mercaptoethanol, 45 μM ascorbic acid) with 100 ng/ml Activin A (only on day 1), 10 ng/ml FGF-2, 100 ng/ml BMP4 and 100 ng/ml VEGF for 7 days. Ectoderm was induced using Neural induction medium (DMEM/F12, 0.4% B27, 1% N2, 1% GlutaMAX, 1% MEM Non-essential aminoacids) with 1 μM LDN193189 for 2 days as iPSC colonies, and 2 μM SB431542 and 3 μM CHIR99021 for 7 days as embryoid bodies. cDNA from each culture was mixed in a 1:1:1 ratio to analyse 1 μg by TaqMan hPSC Scorecard (ThermoFisher) as per the manufacturer's instructions.

STR analysis

STR analysis of 18 locations was performed at Garvan Molecular Genetics Institute (Darlinghurst, Australia).

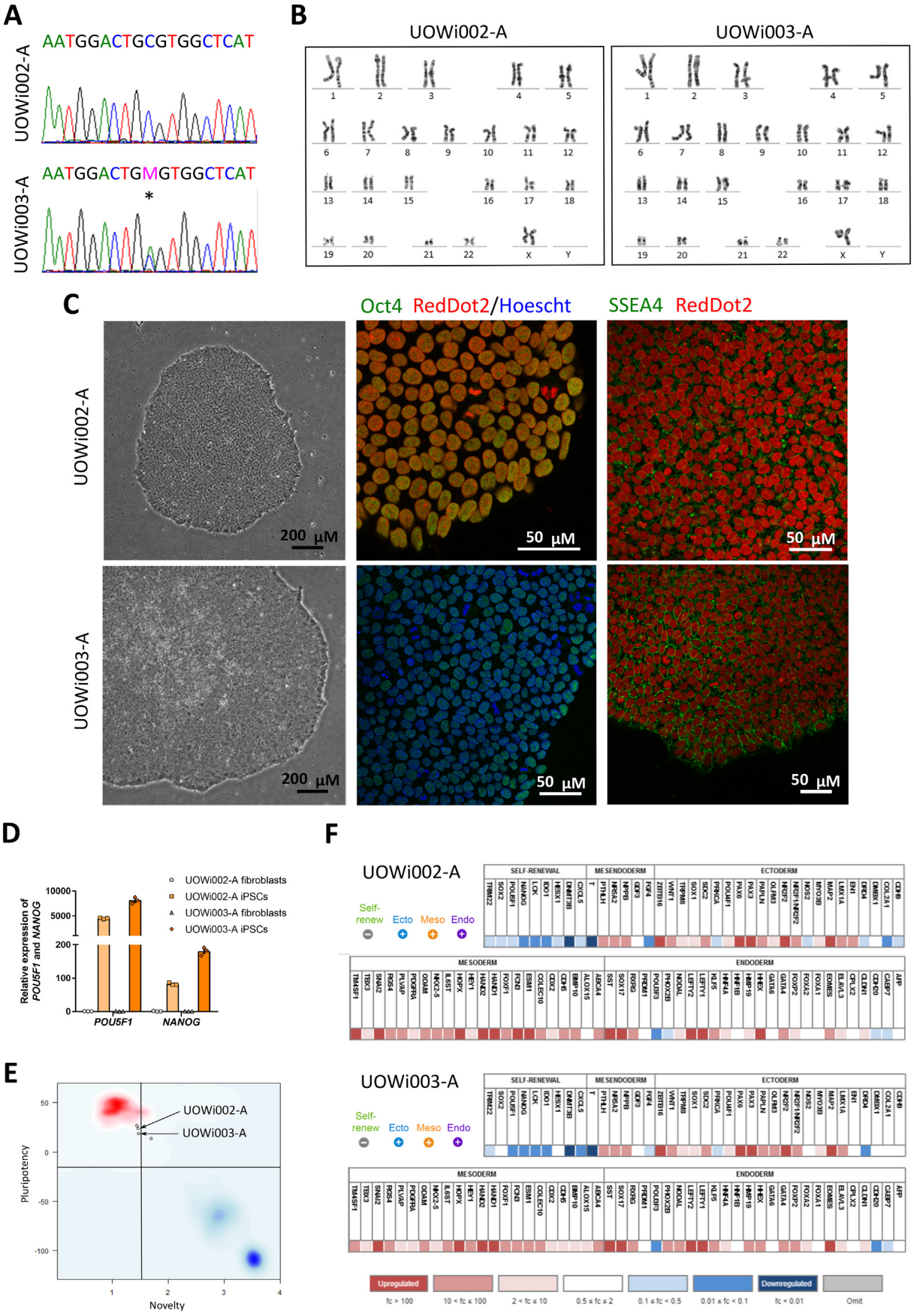

Fig. 1. Characterization of iPSC lines UOWi002-A and UOWi003-A.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1C
Phenotype	Immunocytochemistry	Expression of pluripotency markers Oct4 and SSEA-4	Fig. 1C
	RT-qPCR	Cells express *POU5F1 and NANOG*	Fig. 1D
Genotype	Karyotype (G-banding) and resolution	46XX, resolution: 400 bph	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 18 sites tested, matched	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Yes – mutation present in UOWi003-A and absent in UOWi002-A	Fig. 1A
	Southern Blot or WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Luminescence, negative	Supplementary file 2
Differentiation potential	PluriTest	Pluripotency score: UOWi002-A = 24.16 UOWi003-A = 18.64 Novelty score: UOWi002-A = 1.46 UOWi003-A = 1.47	Fig. 1E
	TaqMan hPSC scorecard	Passed	Fig. 1F
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

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	OCT4	1:500	Stemcell Technologies Cat# 01550, RRID: AB_1118539
	SSEA4	1:200	Abcam Cat# ab16287, RRID: AB_778073
Secondary antibodies	Alexa Fluor 488 Goat anti-mouse IgG (H + L)	1:1000	Thermofisher Scientific Cat# A11001, RRID: AB_2534069

Primers

	Target	Forward/reverse primer (5′-3′)
Sequencing	*PSEN1*	Forward: GGGAGCCATCACATTATTC Reverse: CCTGTGACAAACAAATTATCAG
Pluripotency genes (qPCR)	*NANOG*	Forward: CCAGAACCAGAGAATGAAATC Reverse: TGGTGGTAGGAAGAGTAAAG
	POU5F1	Forward: GATCACCCTGGGATATACAC Reverse: GCTTTGCATATCTCCTGAAG
House-keeping genes (qPCR)	*GAPDH*	Forward: GAGCACAAGAGGAAGAGAGAGACCC Reverse: GTTGAGCACAGGGTACTTTATTGATGGTACATG
	HPRT1	Forward: TGACACTGGCAAAACAATGCA Reverse: GGTCCTTTTCACCAGCAAGCT

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.08.006.

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