



Lab Resource: Genetically-Modified Multiple Cell Lines



Generation and characterisation of four multiple sclerosis iPSC lines from a single family

Alastair J. Fortune, Bruce V. Taylor, Jac C. Charlesworth, Kathryn P. Burdon, Nicholas B. Blackburn, Jessica L. Fletcher, Ashish Mehta, Kaylene M. Young*

Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

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ABSTRACT

Multiple sclerosis (MS) is a complex neuroinflammatory/degenerative disease of the central nervous system (CNS) that results in the formation of demyelinated lesions and axon degeneration. MS aetiology is complex, with genetics estimated to account for ~48% of MS risk (International Multiple Sclerosis Genetics Consortium, 2019). Despite this, families with a high incidence of MS are rare. We have generated four induced pluripotent stem cell (iPSC) lines from individuals with relapsing-remitting and secondary progressive MS within a single family. The generation of disease-specific iPSC lines from multiple members of a single family will facilitate MS genetic and functional studies.

1. Resource utility

MS aetiology has been difficult to elucidate as MS risk stems from interacting environmental, lifestyle and genetic factors. Affected and unaffected closely related family members grow up in a shared environment, likely share lifestyle / environmental factors and provide a unique opportunity to study MS genetic aetiology.

2. Resource Table 1

Unique stem cell lines identifier	MNZTASi002-A (https://hpscereg.eu/cell-line/MNZTASi002-A) MNZTASi003-A (https://hpscereg.eu/cell-line/MNZTASi003-A) MNZTASi004-A (https://hpscereg.eu/cell-line/MNZTASi004-A) MNZTASi005-A (https://hpscereg.eu/cell-line/MNZTASi005-A)
Alternative name(s) of stem cell lines	MS_0001 MS_0002 MS_0003 MS_0006
Institution	Menzies Institute for Medical Research, University of Tasmania
Contact information of distributor	Kaylene Young Kaylene.young@utas.edu.au
Type of cell lines	iPSC

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Origin	Human
Additional origin info required for human ESC or iPSC	Age: 52(F), 55(F), 30(F), 54(M) Ethnicity: Caucasian (All)
Cell Source	Peripheral mononuclear blood cells (PBMCs)
Clonality	Clonal
Associated disease	Relapsing remitting and secondary progressive multiple sclerosis
Gene/locus	N/A
Date archived/stock date	Stored at Menzies Institute for Medical Research, Dec 2021
Cell line repository/bank	Cell lines are registered at https://hpscereg.eu/cell-line/MNZTASi002-A ; https://hpscereg.eu/cell-line/MNZTASi003-A ; https://hpscereg.eu/cell-line/MNZTASi004-A ; https://hpscereg.eu/cell-line/MNZTASi005-A but not deposited.
Ethical approval	University of Tasmania Human Research Ethics Committee H0016915

3. Resource details

MS is a chronic autoimmune neuroinflammatory disease of the CNS that affects over 2.8 million people globally (Multiple Sclerosis International Federation, 2020). People with MS follow either a relapsing-remitting (RRMS), secondary progressive (SPMS) or primary

* Corresponding author at: Menzies Institute for Medical Research, 17 Liverpool St, Hobart, Tasmania 7000, Australia.

E-mail address: kaylene.young@utas.edu.au (K.M. Young).

progressive (PPMS) disease course. RRMS is the most common, experienced at onset by 85% of people with MS and is characterised by relapses in disease severity that are followed by periods of remission in which symptoms resolve or improve. Overtime, most people with RRMS transition to the SPMS disease course and experience a continual worsening of disease severity without relapses. PPMS affects ~ 15% of people with MS and is associated with a progressive worsening of symptoms from disease onset without relapses. The average age of MS diagnosis is 32 years and MS more commonly affects females (~3:1 ratio), but the reason for this is unknown.

The cell type/s or signalling pathways involved in MS initiation are poorly understood, but stem from a combination of genetic, environmental and lifestyle factors. Estimates of the genetic component of MS (i.e. inherited risk) vary widely from study to study, ranging from 19.2% to 76%. Indeed 15–20% of people with MS have a family history of the disease, and first-degree relatives of people with MS have an estimated 3% lifetime risk of developing MS themselves. Multicase families with 3 or more affected close relatives are rare, but present important opportunities to study MS. Further, iPSC lines derived from people with MS enable MS pathophysiology, particularly intrinsic cellular phenotypes resulting from the underlying genetics, to be studied in cell lines with a relevant genetic background (Fortune et al., 2022). We generated 4 MS iPSC lines from individuals spanning 2 generations of a single family. The 4 iPSC lines were generated from 3 females and 1 male, and 2 RRMS and 2 SPMS cases.

Peripheral mononuclear blood cells (PBMCs) were isolated from the 4 affected family members and reprogrammed into iPSCs using a Sendai virus that encoded OCT3/4, SOX2, KLF4 and c-MYC. 6 clones were manually selected and cultured from each person, but only one clone was characterised and validated as detailed in Table 1. All 4 iPSC lines had a typical embryonic stem cell-like morphology and a high nuclear:cytoplasmic ratio (Fig. 1A). Flow cytometry (Fig. 1B) and immunohistochemistry (Fig. 1C) for the pluripotency marker, OCT4, revealed high levels of expression in all cell lines, similar to that of a previously published iPSC line (Mehta et al., 2021). All iPSC lines expressed the pluripotency markers *POU5F1*, *NANOG* and *SOX2* by PCR (Fig. 1D). Embryoid bodies were allowed to spontaneously form for each cell line and immunohistochemistry confirmed expression of a marker denoting each germ layer: NESTIN (ectoderm), GATA4 (mesoderm) and AFP (endoderm) (Fig. 1E). All cell lines eliminated the Sendai virus by passage 13 (Fig. 1F) and had karyotypically normal karyograms (Fig. 1G). Finally, iPSC line identities were confirmed by short tandem repeat analysis and all iPSC lines were mycoplasma negative.

4. Materials and methods

4.1. Ethics statement

The generation and characterisation of these hiPSC lines was approved by the University of Tasmania Human Research Ethics Committee (H0016915). The study was performed according to the approved ethics protocol, including the receipt of informed consent.

4.2. PBMC collection and reprogramming

4–5 ml of blood was collected from each donor into EDTA Vacutainer blood collection tubes. PBMCs were isolated using a Lymphoprep (StemCell Technologies) density gradient and cultured in StemSpan II medium with Erythroid expansion supplement (StemCell Technologies). Reprogramming was performed using the Cytotune 2.0 Sendai Virus Kit (ThermoFisher Scientific). Colonies exhibiting hiPSC-like morphology were manually selected 3–4 weeks post-transduction and were maintained on Matrigel and mTeSR1™ plus (StemCell Technologies) with a 7-day passage cycle as described previously (Mehta et al., 2018).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Immunocytochemistry	Positive staining of pluripotency marker, OCT4; counterstained with DAPI	Fig. 1C
	Flow cytometry	Positive for pluripotency marker Oct4	Fig. 1B
Genotype	RT-PCR	Expression of endogenous <i>POU5F1</i> , <i>NANOG</i> and <i>SOX2</i>	Fig. 1D
	Karyotype (G-banding) and resolution	MS1 46XY, MS2 46XX, MS3 46XX, MS6 46XX, 20 metaphases each Resolution: 550	Fig. 1G
Identity	Microsatellite PCR	Not performed	Not performed
	STR analysis	10 loci analysed, all matching	Submitted in archive with journal
Mutation analysis	Sequencing	Not performed	Not performed
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Submitted in archive with journal
	Sendai Virus	Sendai Virus testing by RT-PCR. Eliminated with repeat passaging	Fig. 1F
Differentiation potential	Embryoid body formation and immunocytochemistry	Positive staining for NESTIN (ectoderm), GATA4 (mesoderm) and AFP (endoderm); counterstained with DAPI	Fig. 1E
	RT-PCR	Not performed	Not performed
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	All negative	Data not shown but available from authors
Genotype additional info	Blood group genotyping	Not performed	Not performed
	HLA tissue typing	Not performed	Not performed

4.3. *In vitro* differentiation assay

Cells were differentiated *in vitro* into embryoid bodies to determine the presence of the three germ layers. Embryoid bodies were cultured in 10% EB medium (DMEM/F12 with GlutaMax and FBS) for 10 days and the medium was changed every three days. EBs were dissociated into a single cell suspension for immunocytochemistry.

4.4. Immunocytochemistry and flow cytometry

Cells were fixed with 4% (w/v) paraformaldehyde for 15 min at ~21 °C, rinsed with PBS and permeabilised with 10% FBS (Sigma) / 0.1% Triton X-100 (Sigma) in PBS for 30 min. Cells were incubated with the primary (4 °C overnight) and secondary (~21 °C for 1 h) antibodies detailed in Table 2. Samples were mounted with Prolong Gold antifade containing DAPI (ThermoFisher Scientific) and images were captured using an UltraView spinning disk confocal microscope attached to a

Figure 1: Generation and characterization of multiple sclerosis hiPSCs from a family

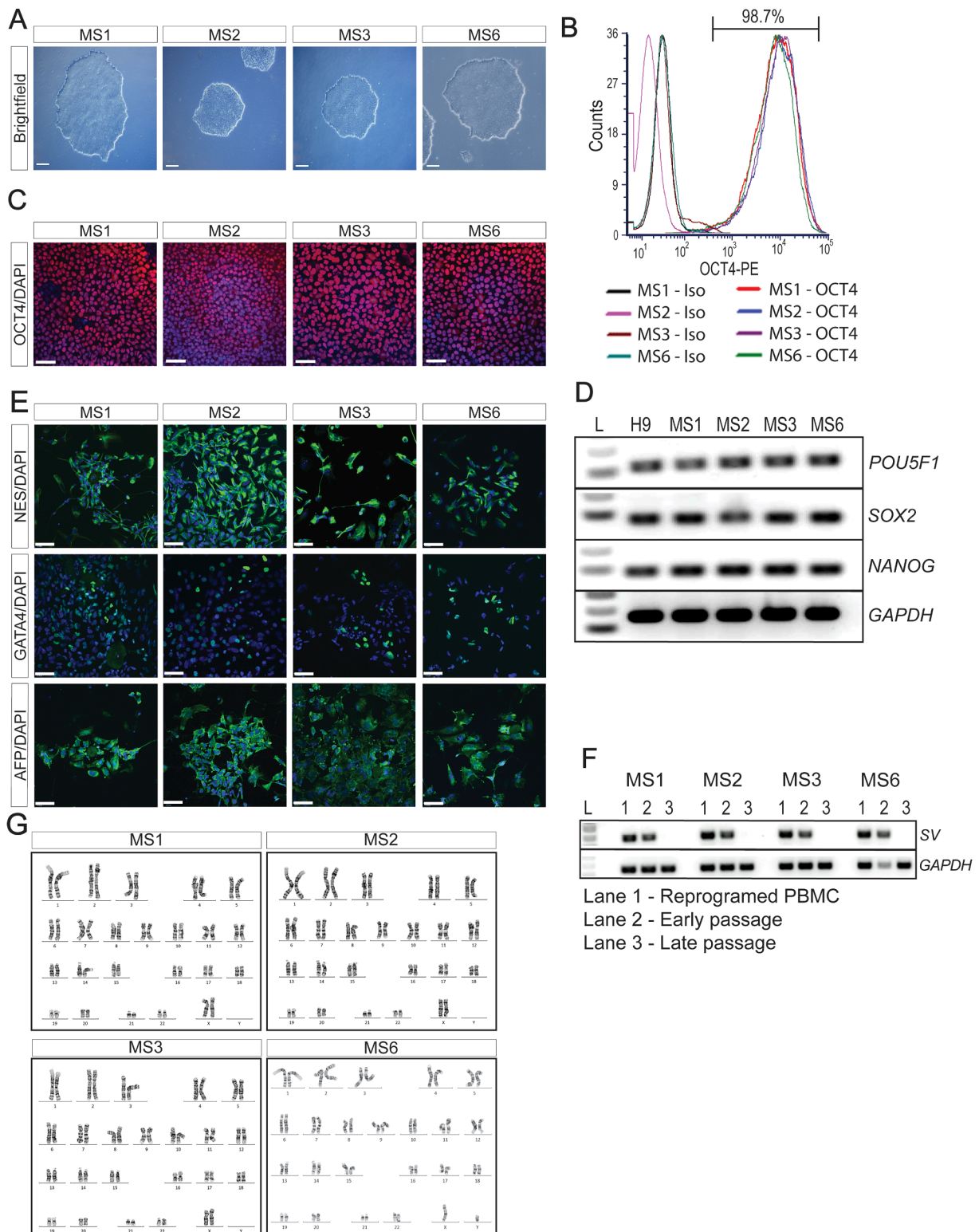


Fig. 1. Generation and characterization of multiple sclerosis hiPSCs from a family.

Nikon Ti Microscope with Velocity Software (Perkin Elmer). For flow cytometry, fixed and permeabilised cells were instead incubated with directly conjugated primary antibody for 1 h at 4 °C (Table 2) and washed in PBS. 10,000 events were acquired on a BD FACSCanto II (BD Biosciences).

4.5. RT-PCR

RNA was isolated using the RNeasy Plus Mini kit (Qiagen). cDNA was generated using the Superscript IV VILO kit (ThermoFisher Scientific) and amplified with SYBR Green Master mix on a Quant Studio 3 (ThermoFisher Scientific) under the following conditions: denaturation

Table 2
Reagents details.

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit IgG Oct-4A	1:100	Cell Signaling Technology Cat. No. 2840, RRID: AB_2167691
Differentiation markers	NESTIN Mouse Ab	1:1600	Cell Signaling Technology Cat. No. 33475, RRID: AB_2799037
	GATA-4 Rabbit mAb	1:400	Cell Signaling Technology Cat. No. 36966, RRID: AB_2799108
Secondary antibodies	Mouse monoclonal IgG ₁ anti-AFP	1:100	R & D Systems, Cat No. MAB1368-SP, RRID: AB_357658
	Alexa 488 Donkey Anti-mouse IgG	1:2000	ThermoFisher Scientific Cat. No. A21202, RRID: AB_141607
	Alexa 488 Donkey anti-rabbit IgG	1:2000	ThermoFisher Scientific Cat. No. A21206, RRID: AB_2535792
Flow cytometry	Alexa 488 Donkey anti-goat IgG	1:2000	ThermoFisher Scientific Cat. No. A11055, RRID: AB_2534102
	Mouse Anti-Oct3/4-BV421	5 µL	BD Bioscience Cat No. 5656644, RRID: AB_2892805

(95 °C, 5 min), amplification (95 °C, 15 s; 60 °C, 30 s; 72 °C, 30 s for 35 cycles) and final extension (72 °C, 5 min). PCR products were visualised by gel electrophoresis (2% (w/v) agarose gel in TBS).

4.6. Elimination of Sendai virus

The presence of Sendai virus (SV) was detected by PCR using cDNA and GoTaq Green master mix (ThermoFisher Scientific) under the following conditions: denaturation (95 °C, 30 s), amplification (95 °C, 30 s; 55 °C, 30 s; 72 °C, 30 s for 30 cycles). Primers used are listed in Table 3. PCR products were visualised by gel electrophoresis.

4.7. Karyotyping and mycoplasma detection

hiPSC lines were karyotyped by G-banding metaphase analysis (Cytogenetics Laboratory, Royal Hobart Hospital, Hobart, Tasmania), and the presence of mycoplasma was assessed using the MycoAlert Mycoplasma detection kit (Lonza Biosciences).

4.8. STR analysis

Genomic DNA was extracted from PBMCs and iPSCs using the DNeasy Kit (Qiagen) and samples were sent to the Australian Genome Research Facility for STR analysis.

Table 3
Primers

Primers	Target	Forward/reverse primer (5'–3')
Sendai virus (SV)	SV, 181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
House-keeping gene	<i>GAPDH</i> , 153 bp	GTGGACCTGACCTGCCCTCT/ GGAGGAGTGGGTGTGCGTGT
Endogenous Pluripotency Markers (RT-PCR)	<i>POU5F1</i> , 113 bp <i>NANOG</i> , 89 bp <i>SOX2</i> , 76 bp	AGTTTGTGCCAGGGTTTTTG/ ACTTCACCTTCCCTCCAACC CTCCATGAACATGCAACCTG/ GAGGAAGATTGACGCCAGTG AAAAATCCCATCACCCACAG/ GCGGTTTTTTCGCTGAGTGT

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.

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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.2022.102828>.

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