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Lab Resource: Single Cell Line

Generation of MNZTASi001-A, a human pluripotent stem cell line from a person with primary progressive multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune and neurodegenerative disease that results in immune cell infiltration of the central nervous system (CNS) and demyelination in young adults. Substantial progress has been made in developing disease modifying therapies for people with relapsing-remitting MS, but options remain limited for people with primary progressive MS (PPMS). PPMS accounts for ~15% of MS diagnoses. Herein, we generated a human induced pluripotent stem cell line (hiPSC) from a person with clinically definite PPMS. This disease-specific hiPSC line will be useful for studying PPMS *in vitro*, allowing the generation of immune and CNS cell types.

1. Resource Table

Unique stem cell line identifier	MNZTASi001-A
Alternative name of stem cell line	MS_0004.2
Institution	Menzies Institute for Medical Research, University of Tasmania
Contact information of distributor	Kaylene Young; kaylene.young@utas.edu.au
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 65 years Sex: Male Ethnicity: Caucasian
Cell source	PBMCs
Clonality	Clonal
Method of reprogramming	Sendai virus (CytoTune 2.0) – Transgenes : Oct, Sox2, Klf4, c-Myc
Genetic modification	No
Type of genetic modification	N/A
Associated disease	Primary progressive multiple sclerosis
Gene/locus	Not determined
Cell line archived	Apr 2021
Ethical approval	University of Tasmania Human Research Ethics Committee H0016915

2. Resources utility

A diagnosis of PPMS requires 1 year of disability progression, the presence of T2-hyperintense lesions in the brain and / or spinal cord, and oligoclonal bands in the cerebral spinal fluid. To study mechanisms underlying the associated immune and CNS pathology, hiPSCs were generated from a person with PPMS.

3. Resource details

MS is the most common non-traumatic neurological disease affecting young adults. It is a complex neuroinflammatory disease that results in the formation of demyelinated lesions within the CNS and axon degeneration (Trapp et al., 1998). In people with MS, neuron damage is most highly associated with irreversible disability accrual, and consequently underpins disease progression. MS symptoms can be varied and present as a seemingly irregular disease course, with the majority of people (~85%) being diagnosed with relapsing-remitting MS and eventually transitioning to secondary progressive MS. A minority of people diagnosed (~15%) show evidence of disability progression from disease onset and are defined as having PPMS. Unlike relapsing-remitting MS, which affects more women, PPMS affects a similar number of men and women. A number of lifestyle, environmental, and genetic risk factors have been identified for MS development and

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Figure 1

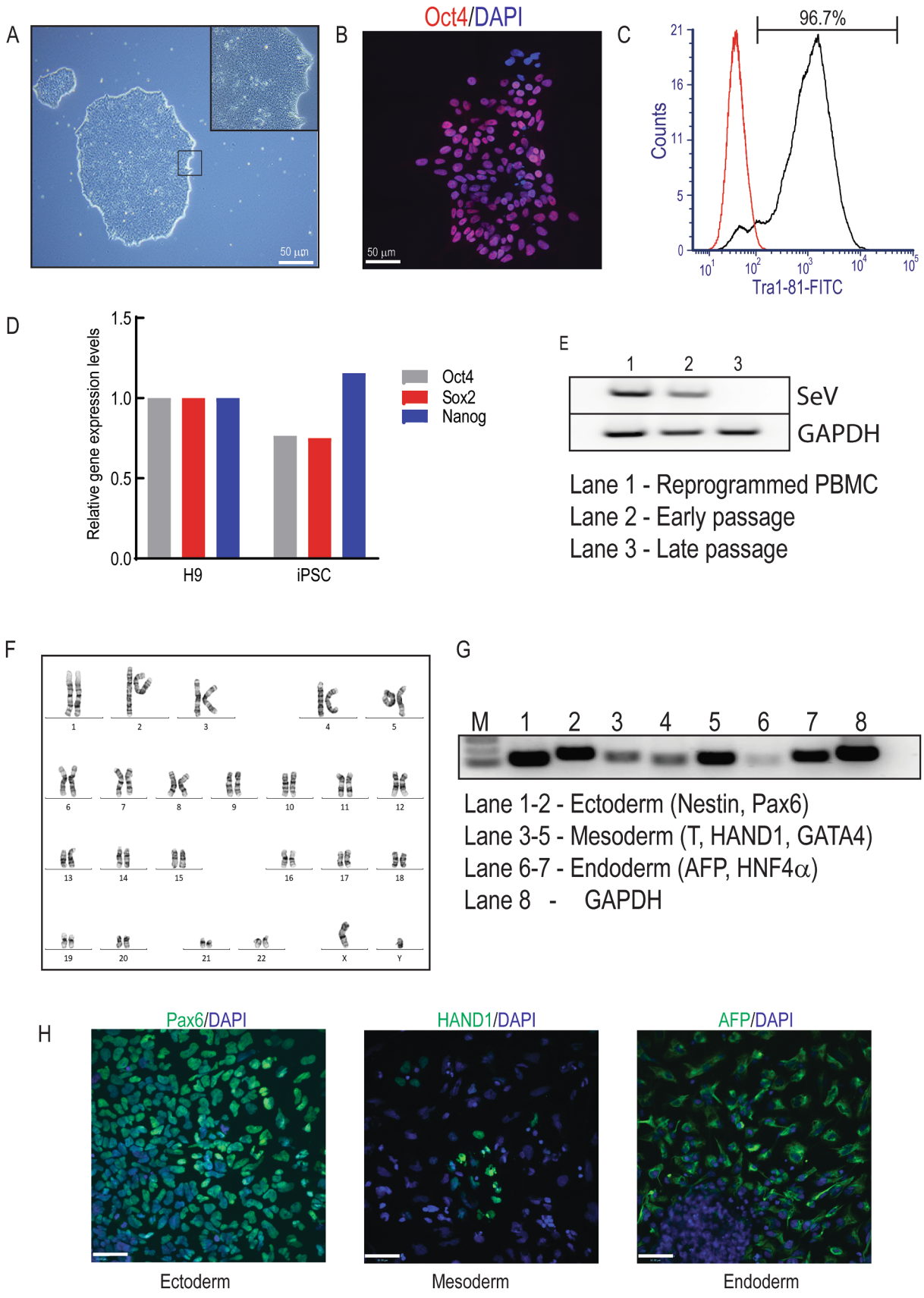

Fig. 1. Characterization of MNZTASi001-A, a hiPSC line.

progression, however, the cause of MS remains unknown.

To study the phenotype and response of immune and CNS cell types that are relevant to MS pathology, in a cell line with a relevant genetic background, we generated iPSCs from a person with PPMS. The 65-year-old male was diagnosed with clinically definite PPMS at aged 39. The diagnosis of PPMS was based on 1 year of disability progression and the presence of T2-hyperintense lesions in the brain and spinal cord (Mcginley et al., 2021).

Human iPSCs were generated from peripheral blood mononuclear cells (PBMCs) using a Sendai virus encoding the reprogramming factors, OCT3/4, SOX2, KLF4 and c-MYC, under feeder-free conditions (Viswanathan et al., 2018). Ten of the resulting clones were manually picked and cultured, and one clone was characterized for this study. MNZTASi001-A had typical human embryonic stem cell-like morphology with a high nuclear to cytoplasmic ratio (Fig. 1**A**). MNZTASi001-A expressed the pluripotent marker, Oct4, by immunocytochemistry (Fig. 1**B**) and 96.7% of the harvested cells were Tra-1–81^{+} by flow cytometry (Fig. 1**C**). By qRT-PCR, MNZTASi001-A also expressed *POU5F1*, *NANOG* and *SOX2* at levels comparable to that of the H9 human embryonic stem cell line (Fig. 1**D**). The MNZTASi001-A cell line is foot-print free, as repeated passaging successfully eliminated the Sendai virus (Fig. 1**E**), and the line is karyotypically normal, based on an evaluation of 20 metaphase spreads at passage 4 (Fig. 1**F**). When MNZTASi001-A was allowed to spontaneously differentiate, forming embryoid bodies, the presence of three germ layers was first demonstrated by the expression of the ectodermal genes *Nestin* and *Pax6*, the mesodermal genes *T*, *HAND1*, and *GATA4*, and the endodermal genes *AFP* and *HNF4a* (Fig. 1**G**), by RT-PCR. We validated the gene expression results by immunocytochemistry, detecting the ectodermal marker, PAX6, the mesodermal marker, HAND1 and the endodermal marker AFP (Fig. 1**H**). The identity of the cell line was verified by short tandem repeat (STR) analysis and the cells were confirmed to be mycoplasma negative prior to cryopreservation.

4. Materials and methods

4.1. Ethics statement

The generation and characterisation of this hiPSC line 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 written informed consent.

4.2. PBMC collection and reprogramming

Blood (4–5 ml) was collected into an EDTA Vacutainer blood collection tube. 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 Kit (Thermofisher Scientific), as per the manufacturer's instructions. 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).

4.3. In vitro differentiation assay

Cells were differentiated *in vitro* into the three germ layers via embryoid body formation. Embryoid bodies were cultured in 10% EB medium (DMEM/F12 with GlutaMax and FBS) for 10 days with the medium changed every third day. Embryoid bodies were collected for RT-PCR or dissociated into single cells for immunocytochemistry.

4.4. Immunocytochemistry and flow cytometry

Cells were fixed with 4% (w/v) paraformaldehyde for 15 min at room temperature, PBS rinsed, and permeabilised with 2% BSA (Sigma) / 0.2% Triton X-100 (Sigma) in PBS for 30 min. Cells were incubated with the primary (4 °C overnight) and secondary (room temperature 1 h) antibodies detailed in Table 1. 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 Nikon Ti Microscope with Volocity 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 1) and washed in PBS, before 10,000 events were acquired on a BD FACSCanto II (BD Biosciences).

Table 1
Characterization and validation of the MNZTASi001-A hiPSC line.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig 1, panel A
Phenotype	Immunocytochemistry	Positive staining of pluripotency marker, OCT4; counterstained with DAPI	Fig. 1, panel B
	Flow cytometry	Positive for pluripotency marker, Tra1-81	Fig. 1, panel C
	qRT-PCR	Expression of endogenous *POU5F1, NANOG, SOX-2*	Fig. 1, panel D
Differentiation potential	Immunocytochemistry	Positive staining for PAX6 (ectoderm), HAND1 (mesoderm), AFP (endoderm), counterstained with DAPI	Fig. 1, panel H
	RT-PCR	Presence of *Nestin*, *Pax6* (Ectoderm), *T*, *GATA4*, *Mesp1* (Mesoderm), *AFP* and *HNF4α* (Endoderm) with *GAPDH* (Housekeeping gene)	Fig. 1, panel G
Genotype	Karyotype (G-banding)	46XY, 20 metaphases	Fig. 1, panel F
Identity	Microsatellite PCR	Not performed	Not available
	STR analysis	10 loci analysed, all matching	Submitted in archive with journal
Mutation analysis	Sequencing	Not performed	Not available
	Southern Blot or whole genome sequencing	Not performed	Not available
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Data not shown but available from authors
	Sendai Virus	Sendai Virus testing by RT PCR. Eliminated with repeat passaging	Fig. 1, panel E
Donor screening	HIV 1 + 2; Hepatitis B; Hepatitis C	All negative	Not shown but available from authors
Genotype additional info	Blood group genotyping	Not performed	Not available
	HLA tissue typing	Not performed	Not available

Table 2
Reagent Details Antibodies used for immunocytochemistry/flow-cytometry.

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit IgG anti-Oct-4A	1:100	Cell Signaling Technology Cat. No. 2840, RRID: AB_2167691
Differentiation markers	Rabbit IgG anti-PAX6	1:200	Cell Signaling Technology Cat. No. 60433, RRID: AB_2797599
	Goat polyclonal IgG anti-HAND1	1:400	R & D Systems, Cat No. AF3168-SP,RRID: AB_2115853
	Mouse monoclonal IgG_1 anti-AFP	1:100	R & D Systems, Cat No. MAB1368-SP, RRID: AB_357658
Secondary antibodies	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
	Alexa 488 Donkey anti-goat IgG	1:2000	Thermofisher Scientific Cat. No. A11055, RRID: AB_2534102
Flow cytometry	PE Mouse IgM, κ Isotype control	5 μL	BD Bioscience Cat No. 5656644, RRID: AB_395960
	PE Mouse anti-human Tra-1–81	5 μL	BD Bioscience Cat No. 5656644, RRID: AB_1645540

4.5. RT-PCR and qRT-PCR

RNA was isolated using a RNeasy Plus Mini kit (Qiagen). cDNA was generated using a Superscript IV VILO kit (Thermofisher Scientific). For germ layer RT-PCR, cDNA was amplified with GoTaq Green master mix (Thermofisher Scientific) under the following condition: denaturation (95 °C, 5 min), amplification (95 °C, 15 s; 58 °C, 30 s; 72 °C, 30 s for 30 cycles) and final extension (72 °C, 5 min). PCR products were visualized by gel electrophoresis [2% (w/v) agarose gel in TBS]. For qRT-PCR, cDNA was amplified with SYBR Green Master mix on a Quant Studio 3 (Thermofisher Scientific) under the following conditions: denaturation (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). Ct values were normalized to *GAPDH* as the reference gene.

4.6. Elimination of Sendai virus

The presence of Sendai virus (SeV) was detected by PCR using cDNA (5 μL, generated from 2 μg of hiPSC RNA) 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 2. PCR products were visualised by gel electrophoresis [2% (w/v) agarose gel in TBS].

4.7. Karyotyping, mycoplasma detection and STR analysis

The iPSC line was karyotyped by G-banding metaphase analysis (Cytogentics Laboratory, Royal Hobart Hospital, Hobart, Tasmania), and the presence of mycoplasma was tested using the MycoAlert Mycoplasma detection kit (Lonza Biosciences), as per the manufacturer's instructions. Genomic DNA was extracted from the iPSCs and PBMCs using DNAEasy Kit (Qiagen) and samples sent to the Australian Genome Research Facility (AGRF, Australia) for STR analysis.

Author contributions

AM, BVT, JC, AC, KB, AH and KMY developed the project. AM and PL carried out the experiments. BVT, JC and KMY obtained the funding. AM performed the statistical analyses and generated the figures. AH provided supervision. AM and KMY wrote the manuscript.

List of primers Primers	Target	Forward/reverse primer (5′–3′)
Sendai virus (SeV)	SeV, 181bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
Ectoderm	*NES*, 111bp	AGGAGAAACAGGGCCTACAGA/ GGAGGGTCCTGTACGTGGC
	PAX6, 147bp	GCGCAGGAGGAAGTGTTTTG/ TCTCAGATTCCTATGCTGATTGGT
Mesoderm	*T, 118bp*	GCGCGAGAACAGCACTACTA/ GACCAAGACTGTCCCCGCTC
	MESP1, 100bp	CGAGTCCTGGATGCTCTCTG/ CCATGAGTCTGGGGACGAGA
	GATA4, 112bp	CGACACCCCAATCTCGATATGTT/ ACAGATAGTGACCCGTCCCA
Endoderm	*AFP, 100bp*	TGTCTGCAGGATGGGGAAAA/ GTTCCAGCGTGGTCAGTTTG
	HNF4α, 128bp	TGCGACTCTCCAAAACCCTC/ TGATGGGGACGTGTCATTGC
House-keeping gene	*GAPDH, 153bp*	GTGGACCTGACCTGCCGTCT/ GGAGGAGTGGGTGTCGCTGT
Endogenous Pluripotency Markers (qRT-PCR)	*POU5F1*	AGTTTGTGCCAGGGTTTTTG/ ACTTCACCTTCCCTCCAACC
	NANOG	CTCCATGAACATGCAACCTG/ GAGGAAGGATTCAGCCAGTG
	SOX2	AAAAATCCCATCACCCACAG/ GCGGTTTTTGCGTGAGTGT
House-keeping gene (qRT-PCR)	*GAPDH*	GTGGACCTGACCTGCCGTCT/ GGAGGAGTGGGTGTCGCTGT

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.2021.102568.

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