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Lab Resource: Multiple Cell Line

Generation of seven iPSC lines from peripheral blood mononuclear cells suitable to investigate Autism Spectrum Disorder

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A B S T R A C T

We have generated and characterized seven human induced pluripotent stem cell (iPSC) lines derived from peripheral blood mononuclear cells (PBMCs) from a single family, including unaffected and affected individuals clinically diagnosed with Autism Spectrum Disorder (ASD). The reprogramming of the PBMCs was performed using non-integrative Sendai virus containing the reprogramming factors POU5F1 (OCT4), SOX2, KLF4 and MYC. All iPSC lines exhibited a normal karyotype and pluripotency was validated by immunofluorescence, flow cytometry and their ability to differentiate into the three embryonic germ layers. These iPSC lines are a valuable resource to study the molecular mechanisms underlying ASD.

Resource utility

ASD is highly heritable (> 80%), therefore iPSC lines from unaffected and affected individuals from a single family provides a valuable pre-clinical model to understand the genetics and molecular pathways contributing to ASD. Understanding the biological mechanisms underlying the development of ASD will enable tailored treatment strategies.

Resource details

Autism Spectrum Disorder (ASD) is a highly heritable neurodevelopmental disorder defined by deficits in social communication and repetitive or stereotyped behaviours with restricted interests (Roehr, 2013). Converging lines of evidence suggest the interaction of genetic and environmental factors can mediate milder phenotypes of ASD such as the broader autism phenotype (BAP) (Iossifov et al., 2014). Individuals with ASD with normal cognitive ability such as BAP have a lower rate of *de novo*, likely gene-disrupting mutations, compared to individuals of lower IQ, which suggests that genetic variants may contribute to the milder phenotype (Chang et al., 2015; Chen et al., 2015). Therefore, the analysis of large multiplex families with discordant ASD phenotypes represents an innovative and powerful avenue to study the genetic basis and modifiers of ASD. Furthermore, neuronal cells generated from patient derived iPSCs offer an unprecedented opportunity for *ex vivo* complementary mechanistic studies to further understand ASD neurobiology.

Blood samples were collected from individuals from a family with ASD (manuscript under review) and peripheral blood mononuclear cells (PBMCs) were extracted. Seven human iPSC lines were generated from the PBMCs using the Sendai virus carrying the Yamanaka reprogramming factors POU5F1 (OCT4), SOX2, KLF4 and MYC (Table 1). All iPSC lines displayed typical iPSC morphology, including small and tightly packed cells, high nucleus to cytoplasm ratio and a prominent nucleoli (Fig. 1A). The pluripotency of the cells was verified by immunofluorescence staining using the pluripotency marker OCT4 (Fig. 1B). In

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
ASD2-3.1 (MCRIi011-A)	ASD2-3.1	Male	57	Caucasian	N/A	Broader Autism Phenotype
ASD2-6.4 (MCRIi012-A)	ASD2-6.4	Male	62	Caucasian	N/A	Broader Autism Phenotype
ASD2-7.1 (MCRIi013-A)	ASD2-7.1	Male	26	Caucasian	N/A	Autism Spectrum Disorder
ASD2-9 .4 (MCRIi014-A)	ASD2-9.4	Female	31	Caucasian	N/A	Unaffected
ASD2-11.5 (MCRIi015-A)	ASD2-11.5	Male	24	Caucasian	N/A	Unaffected
ASD2-15.1 (MCRIi016-A)	ASD2-15.1	Male	19	Caucasian	N/A	Autism Spectrum Disorder
ASD2-75.4 (MCRIi017-A)	ASD2-75.4	Female	69	Caucasian	N/A	Unaffected

addition, flow cytometry was used to assess pluripotency using the markers TRA181, SSEA4, CD9 and EPCAM (Fig. 1C). > 80% of viable cells sorted were positive for SSEA-4, TRA-1-81 and CD9. All iPSC lines were able to form embryoid bodies *in vitro*, which expressed markers consistent with development of the three germ layers. Specifically, immunofluorescence staining identified the ectoderm marker MAP2, the mesodermal marker SMA and the endodermal marker SOX17 (Fig. 1D, E and F respectively). All iPSC lines showed a normal karyotype (with 0.5 Mb resolution) and an identical genotype to their corresponding PBMC sample when analysed by array comparative genomic hybridisation (SNP array), confirming that no perturbations in genomic integrity occurred during reprogramming (Supplementary 1). Furthermore all iPSC lines were mycoplasma free. All characterization and validation analyses are summarized in Table 2. In conclusion, we have generated seven iPSC lines that can be used to differentiate into neuronal models to study the molecular mechanisms of ASD and can serve a valuable resource for drug development and disease modelling studies.

Materials and methods

PBMC isolation

Blood samples were collected from seven individuals from a family affected by ASD. These samples consisted of three unaffected individuals, two with ASD and two with BAP. PBMC isolation was performed by diluting the blood 1:2 (vol:vol) in PBS/2% FBS and layered over Lymphoprep (StemCell Technologies) in SepMate™-15 tubes (StemCell Technologies). Samples were then centrifuged at 1200 rcf for 10 min, transferred to a fresh tube, washed with PBS/2% FBS and centrifuged at 300 rcf for 10 min.

iPSC generation

Reprogramming of PBMCs into iPSCs were performed using the Cytotune-iPS 2.0 Sendai Reprograming kit which included the four Yamanaka reprogramming factors POU5F1 (OCT4), SOX2, KLF4 and MYC (ThermoFisher Scientific). Transduced cells were plated on culture dishes seeded with irradiated mouse embryonic fibroblasts (MEFs), and maintained in Knockout DMEM/20% Knockout serum replacer (ThermoFisher Scientific) supplemented with 50 ng/mL of FGF2 (Costa et al., 2008). iPSC colonies were mechanically isolated and expanded as previously described (Costa et al., 2008). Subsequently, the lines were adapted to feeder free bulk culture on plates coated with Vitronectin (Stemcell technologies) in Essential 8 medium (ThermoFisher Scientific) for continued culture. All cells were cultured at 37 °C with 10% CO_2.

Mycoplasma testing

The absence of mycoplasma contamination in the iPSC lines were confirmed by PCR using a commercial service provider, Cerberus Sciences (Adelaide, Australia).

Karyotyping

Karyotyping of the PBMCs and iPSC lines was performed using the Infinium CoreExome-24 v1.1 SNP array. The passage number for each iPSC line that was karyotyped are as follows: ASD2 3.1 P3 + 5, ASD2 6.4 P3 + 5, ASD2 7.1 P3 + 4, ASD2 9.4 P3 + 5, ASD2 11.5 P4 + 3, ASD2 15.1 P4 + 4 and ASD2 75.4 P4 + 4. The data was compared to the human reference sequence hg19/GRCh37 (Feb 2009) and all lines showed a normal karyotype. SNP array comparisons of PBMC and iPSC lines were performed using SNPDuo comparative analysis (http://pevsnerlab.kennedykrieger.org/SNPduo/). No differences were detected between the original PBMC sample and its corresponding iPSC line.

Flow cytometry

iPSCs were harvested and filtered through a cell-strainer cap fettle to a FACs tube. Cells were then incubated with conjugated antibodies for 15 min on ice (Table 3), and stained with Propidium Iodide (Sigma) to identify dead cells prior to acquisition (LSRFortessa, BD Bioscience).

Embryoid Body (EB) formation

EBs were formed by self-aggregation. Briefly, iPSCs were seeded in ultra-low adherence 96 well plates and cultured in EB medium containing E8 media (Stemcell technologies), 0.02% Gentamicin (Gibco/Invitrogen) and.

0.5% polyvinyl alcohol (PVA) (Sigma). After 24 h, the cells were cultured in E8 medium containing 0.02% gentamicin and media was changed every 2 days for 3 weeks. After 3 weeks, the EBs were plated onto gelatin-coated glass coverslips and cultured in E8 medium containing 0.02% gentamicin for 2 weeks.

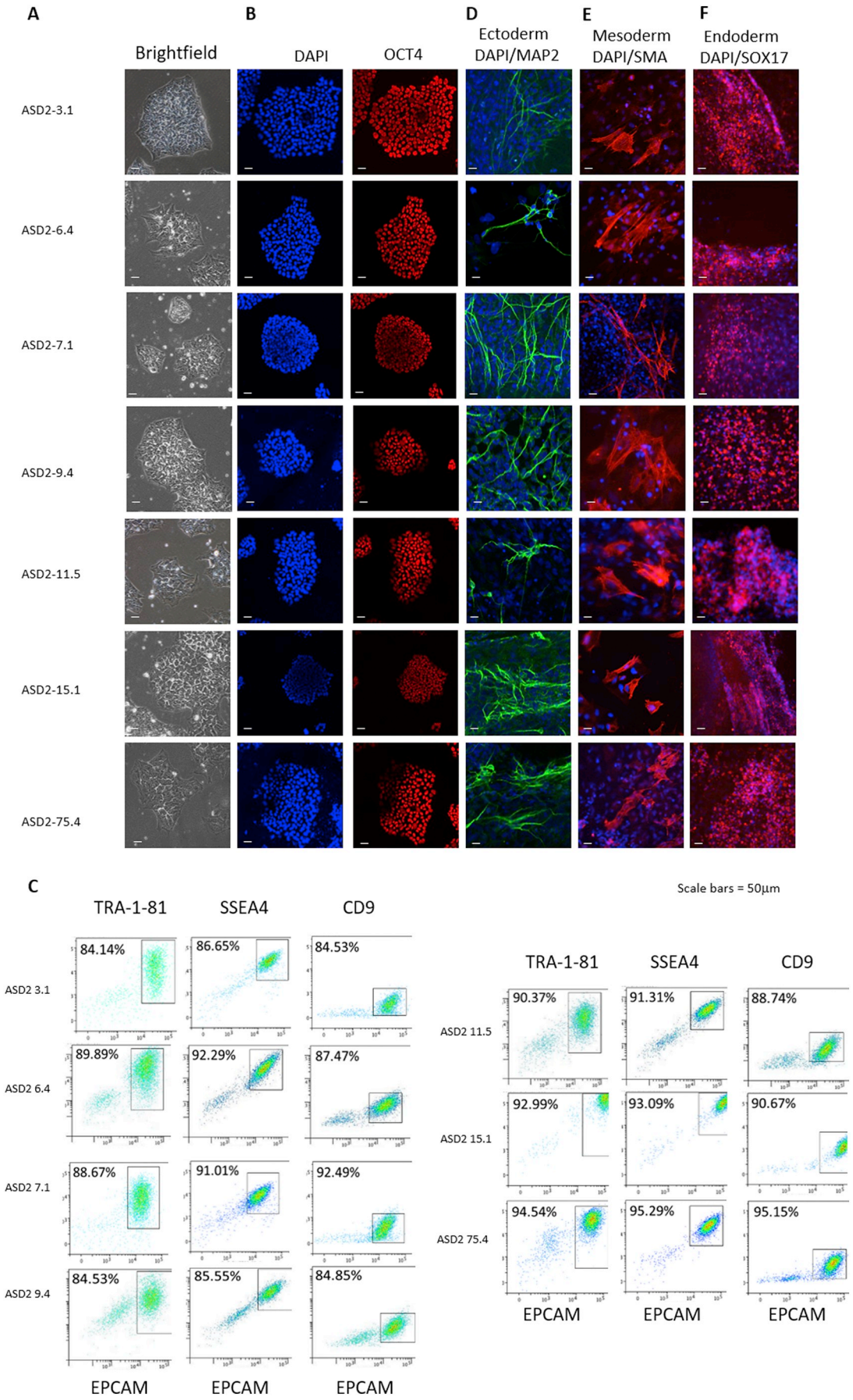
A
B
D
E
F
Brightfield
DAPI
OCT4
Ectoderm
DAPI/MAP2
Mesoderm
DAPI/SMA
Endoderm
DAPI/SOX17
ASD2-3.1
ASD2-6.4
ASD2-7.1
ASD2-9.4
ASD2-11.5
ASD2-15.1
ASD2-75.4
C
Scale bars = 50μm
TRA-1-81
SSEA4
CD9
ASD2 3.1
84.14%
86.65%
84.53%
ASD2 6.4
89.89%
92.29%
87.47%
ASD2 7.1
88.67%
91.01%
92.49%
ASD2 9.4
84.53%
85.55%
84.85%
EPCAM
ASD2 11.5
90.37%
91.31%
88.74%
ASD2 15.1
92.99%
93.09%
90.67%
ASD2 75.4
94.54%
95.29%
95.15%

Fig. 1.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunofluorescence)	Oct4	Fig. 1B
	Quantitative analysis (Flow cytometry)	TRA-1-81: > 84% SSEA4: > 85% CD9: > 84%	Fig. 1C
Genotype	Karyotype (SNP array)	arr(1 − 22)x2,(XY)x1 (resolution 0.50 Mb)	Submitted in archive with journal
Identity	Genetic Analysis	SNPduo comparative analysis performed to compare parental and derived clones	Submitted in archive with journal
		Identical genotypes (> 99.9%) for the entire genome, indicating cell lines are from the same individual	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Data with author
Differentiation potential	Embryoid body formation (immunofluorescence)	Ectoderm: MAP2 Mesoderm: SMA Endoderm: SOX17	Fig. 1 panel 1D, 1E, 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-cytometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-Oct-4A monoclonal antibody (C30A3)	1:400	Cell Signaling Technology Cat#2840 RRID:AB_2167691
Pluripotency Markers	Mouse anti-SSEA4 monoclonal antibody	1:400	Millipore Cat#MAB4304 RRID:AB_177629
Pluripotency Markers	Alexa Fluor 647 anti-human TRA-1-81 antibody	1:100	BioLegend Cat# 330706, RRID:AB_1089242
Pluripotency Markers	PE conjugated anti-human CD326 (EPCAM) antibody, Clone EBA-1	1:30	BD Biosciences Cat# 347198, RRID:AB_400262
Pluripotency Markers	PE/Cy7 anti-human SSEA-4 antibody	1:100	BioLegend Cat# 330420, RRID:AB_2629631
Pluripotency Markers	Mouse Anti-CD9 Monoclonal Antibody, FITC Conjugated, Clone M-L13	1:10	BD Biosciences Cat# 555371, RRID:AB_395773
Differentiation Markers	Unconjugated anti-bovine MAP2 (2a/2b) antibody, Clone AP-20	1:400	Sigma-Aldrich Cat# M1406, RRID:AB_477171
Differentiation Markers	Goat anti SOX17 polyclonal Antibody	1:50	Santa Cruz Biotechnology Cat# sc-17,355, RRID:AB_2239898
Differentiation Markers	Mouse anti-SMA Monoclonal Antibody	1:25	Agilent Cat# M0851, RRID:AB_2223500
Secondary antibodies	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:1000	Molecular Probes Cat# A-11029, RRID:AB_138404
Secondary antibodies	Goat Anti-Chicken IgG (H + L) Antibody, Alexa Fluor 488 Conjugated	1:1000	Molecular Probes Cat# A-11039, RRID:AB_142924
Secondary antibodies	Goat Anti-Mouse IgG (H + L) Highly Cross-adsorbed Antibody, Alexa Fluor 594 Conjugated	1:1000	Molecular Probes Cat# A-11032, RRID:AB_141672
Secondary antibodies	Donkey anti-Goat IgG (H + L) Cross-Adsorbed Secondary Antibody, DyLight 594	1:1000	Thermo Fisher Scientific Cat# SA5–10088, RRID:AB_2556668

Primers

Target	Forward/Reverse primer (5′-3′)
N/A	N/A

Immunofluorescence (IF)

Cells were fixed with 4% Paraformaldehyde (PFA) for 10 min at room temperature, permeabilized in 0.2% Triton X-100 (Sigma) for 10 min at room temperature and blocked in 20% Goat Serum (Life Technologies) for 60 min at room temperature. Cells were then incubated with primary antibodies at 4 °C overnight, followed by secondary antibodies for 60 min at room temperature (Table 3). The coverslips were mounted on slides with mounting media containing DAPI (VectorLabs). Images were captured with an LSM 780 confocal microscope running Zen Black software and an Axio Observer.Z1 microscope with an Axiocam 506 mono camera running Zen Blue software (Carl-Zeiss).

Key resources table

Unique stem cell lines identifier	MCRIi011-A MCRIi012-A MCRIi013-A MCRIi014-A MCRIi015-A MCRIi016-A MCRIi017-A
Alternative names of stem cell lines	ASD2-3.1 (MCRIi011-A) ASD2-6.4 (MCRIi012-A) ASD2-7.1 (MCRIi013-A) ASD2-9.4 (MCRIi014-A) ASD2-11.5 (MCRIi015-A) ASD2-15.1 (MCRIi016-A) ASD2-75.4 (MCRIi017-A)
Institution	Murdoch Children's Research Institute, Parkville, VIC, Australia
Contact information of distributor	Dr Paul Lockhart Paul.lockhart@mcri.edu.au
Type of cell lines	iPSC
Origin	Human
Cell Source	PBMC
Clonality	Clonal
Method of reprogramming	Transgene from Sendai Virus
Multiline rationale	Same condition non-isogenic cell lines
Gene modification	No
Type of modification	N/A
Associated disease	Autism Spectrum Disorder
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	1/2017
Cell line repository/bank	N/A
Ethical approval	This study was approved through the Human Research Ethics Committee of the Royal Children's Hospital (28097), Victoria, Australia

Declaration of Competing Interest

None.

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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.2019.101516.

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