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Derivation of two iPSC lines from a sporadic ASD patient (NUIGi033-A) and a paternal control (NUIGi034-A)

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ABSTRACT

Hundreds of rare risk factors have been identified for ASD, however, the underlying causes for ~70% of sporadic cases are unknown. Sporadic ASD models are thus essential for validating phenotypic commonality and drug suitability to the majority of patients. Here, we derived induced pluripotent stem cells (iPSCs) from one sporadic ASD child and one paternal control, using non-integrating Sendai viral methods. The iPSCs strongly expressed pluripotency markers and could be differentiated into three germ layers. Their normal karyotype was validated by genome SNP array. The availability of sporadic ASD-derived iPSCs offers an opportunity for phenotypic comparison with genetic ASD models.

Resource Table

Unique stem cell lines identifier	NUIGi033-A (ASD012-Q) NUIGi034-A (ASDC012-AJ)
Alternative names of stem cell lines	NUIGi033-A (ASD012-Q) NUIGi034-A (ASDC012-AJ)
Institution	Regenerative Medicine Institute, National University of Ireland Galway, H91 TK33 Galway, Ireland
Contact information of distributor	*Sanbing Shen sanbing.shen@nuigalway.ie*
Type of cell lines	Induced pluripotent stem cells (iPSCs)
Origin	Human
Cell Source	Dermal Fibroblasts
Clonality	Clonal
Method of reprogramming	Integration-free Sendai Virus expressing OCT4, SOX2, c-MYC, KLF4
Multiline rationale	N/A
Gene modification	N/A
Type of modification	N/A
Associated disease	Autism spectrum disorder (ASD)
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	6/2018
Cell line repository/bank	Regenerative Medicine Institute, National University of Ireland, Galway

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Ethical approval	This study has been approved Galway University Hospitals Clinical Ethics Committee (C.A.750). Patient gave their written informed consent for skin biopsy donation.

1. Resource utility

The sporadic ASD iPSCs offer an opportunity for identifying common phenotypes with genetic ASD models and testing drug suitability for most ASD patients. The healthy paternal iPSCs offer a familial control to minimize genetic background effects and an opportunity to create isogenic lines for other diseases with minimal genetic polymorphisms.

2. Resource details

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder associated with a spectrum of core clinical symptoms and an array of central and peripheral comorbidities [Reilly et al., 2017]. ASD is known to have a strong genetic component and hundreds of rare risk factors have been identified [Pinto et al., 2014; Al Shehhi et al., 2019]. However, the underlying genetic causes for approximately 70% of ASD cases (termed sporadic) are unknown [O'Roak et al., 2012]. ASD affects throughout all lifespan of patients, from early childhood till adulthood, with no cure. Life expectancy of these patients is on average 10 years lower than the general public. Drug development studies have been hampered by the complexity of hundreds of rare risk factors as well as the lack of proper human ASD disease models. Creation of a large number of sporadic ASD models are essential to validate the commonality of disease phenotype found in genetic cases of ASD and to test leading therapeutic molecules for the suitability to the majority of ASD patients. Meanwhile, the healthy control iPSCs with close genetic background match are essential for phenotypic characterization including 2D neuronal and 3D organoid disease modeling [Lancaster et al., 2013]. Control iPSCs are also needed for creating isogenic iPSC lines of known genetic defects for different diseases with minimal genetic difference to overcome the common challenge of iPSC heterogeneity.

The patient described has been diagnosed with ASD at 2-year and 8-month with a mild learning disability. There is no significant medical history on either the paternal or maternal side of the family. The patient has two younger female siblings aged 4- and 1-year, respectively who are normally developed.

The iPSCs were derived from dermal fibroblasts at passage 4 and displayed typical embryonic stem cell-like morphology, growing in tightly packed colonies with no obvious cell boundary, but a small cell body and a large nucleus/cytoplasm ratio (Fig. 1A). Their pluripotency was confirmed by high alkaline phosphatase activity (Fig. 1B), positive immunoreactivity of pluripotency markers OCT4, SSEA4, SOX2 and TRA-1-81 (Fig. 1C), and abundant mRNA expression of endogenous OCT4, SOX2 and NANOG genes (Fig. 1E). They were able to form embryoid bodies and spontaneously differentiate into three embryonic germ layers, with positive immunoreactivity for endoderm marker α-fetoprotein (AFP), mesoderm maker α-smooth muscle actin (α-SMA), and ectodermal marker βIII-tubulin (TUJ1) (Fig. 1D).

We carried out whole genome SNP array and did not identify specific/consistent CNVs in the sporadic proband or healthy paternal controls (Table 1, Fig. 1G). However, SNP array could not detect balanced rearrangements and low- level mosaicism. The iPSCs were confirmed to be free of transgene integration (Fig. 1F), or mycoplasma contamination (Supplementary Fig. 1), by RT-PCR and PCR, respectively. The full characterization can be viewed in Table 2 and Fig. 1. The sporadic ASD iPSCs and the paternal healthy control will become valuable resources for investigating molecular mechanisms of ASD and drug validation.

3. Materials and methods

3.1. Cell reprogramming

Skin fibroblasts were cultured at 37 °C, 5% CO2, in the DMEM high glucose (Gibco), supplemented with, 10% FBS (Sigma-Aldrich), 1% penicillin-streptomycin and 1% MEM NEAA solution (Gibco). Cytotune™-iPS 2.0 Sendai Reprogramming kit (ThermoFisher Scientific, Cat. A16518) were used for reprogramming fibroblasts at P4/5 into iPSC. Single iPSC colonies were picked up and expanded by Gentle Cell Dissociation Reagent (STEMCELL) on Geltrex™-coated 6-well plates in Essential 8™ medium (Gibco) at 37 °C, 5% CO2.

3.2. Pluripotency validation

Alkaline Phosphatase Staining Kit II (Stemgent) was used for detecting Alkaline phosphatase activity of iPSCs. For immunofluorescence staining, iPSCs were fixed in 4% PFA for 20 min, permeabilized with 0.1% Triton X-100 (Sigma) for 15 min, and blocked for 1 h in 1% BSA-DPBS, and incubated with primary antibodies against OCT4, SSEA4, SOX2 or TRA-1-81 (Table 2) at 4 °C overnight. Alexa Fluor 488- or 555-conjugated secondary antibodies (Cell Signaling Technology) were then applied to visualize cells, and cell nuclei were labeled with Hoechst 33342 (Life Technologies). Slides were imaged using a Confocal Microscope (Olympus FluoView 1000 system). To detect expression of endogenous pluripotency genes, qRT-PCR was performed with the StepOne Plus Real Time PCR System using Fast SYBR™ Green Master Mix (Applied Biosystems) and specific primers listed in Table 3. The expression levels of OCT4, SOX2 and NANOG were adjusted with an internal control (GAPDH), and then converted to log2 fold of expression over fibroblast mRNA as a negative control.

3.3. Three germ layer differentiation

Embryonic bodies (EB) formation were carried to assess differentiation potential. Detached iPSC colonies by scraping were cultured at 50 rpm on an orbital shaker inside a 37 °C incubator. EBs were cultured in suspension for 5 days in DMEM/F12 medium supplemented with 20% FBS, 1% MEM NEAA, 1% L-Glutamine (200 mM), 0.2% β-mercaptoethanol and 1% penicillin-streptomycin. EBs were then plated onto Geltrex-coated 8-well chambers (iBiDi) for 3-4 weeks and stained with antibodies against AFP, α-SMA and TUJ1 (Table 3).

3.4. Transgene-free confirmation

cDNA were synthesized from total RNA which extracted by RNeasy Mini Kit (Qiagen) using sensiFAST cDNA Synthesis Kit (Sigma-Aldrich). Absence of transgene were detected by PCR analysis by TopTaq® Master Mix (Qiagen) under standard conditions using a set of transgene-specific primers (Table 3).

3.5. Mycoplasma detection

Absence of mycoplasma contamination was confirmed by PCR using the MycoSensor PCR Assay Kit (Agilent), following manufacturer's instructions.

3.6. DNA fingerprinting analysis

six STR loci were PCR amplified to confirm the source of iPSC lines with PCR primers listed in Table 3.

3.7. Karyotyping

The molecular karyotype of iPSCs at passage 20 was analyzed with 990 k SNP array by Beijing Hyslar Biotech Limited Corporation

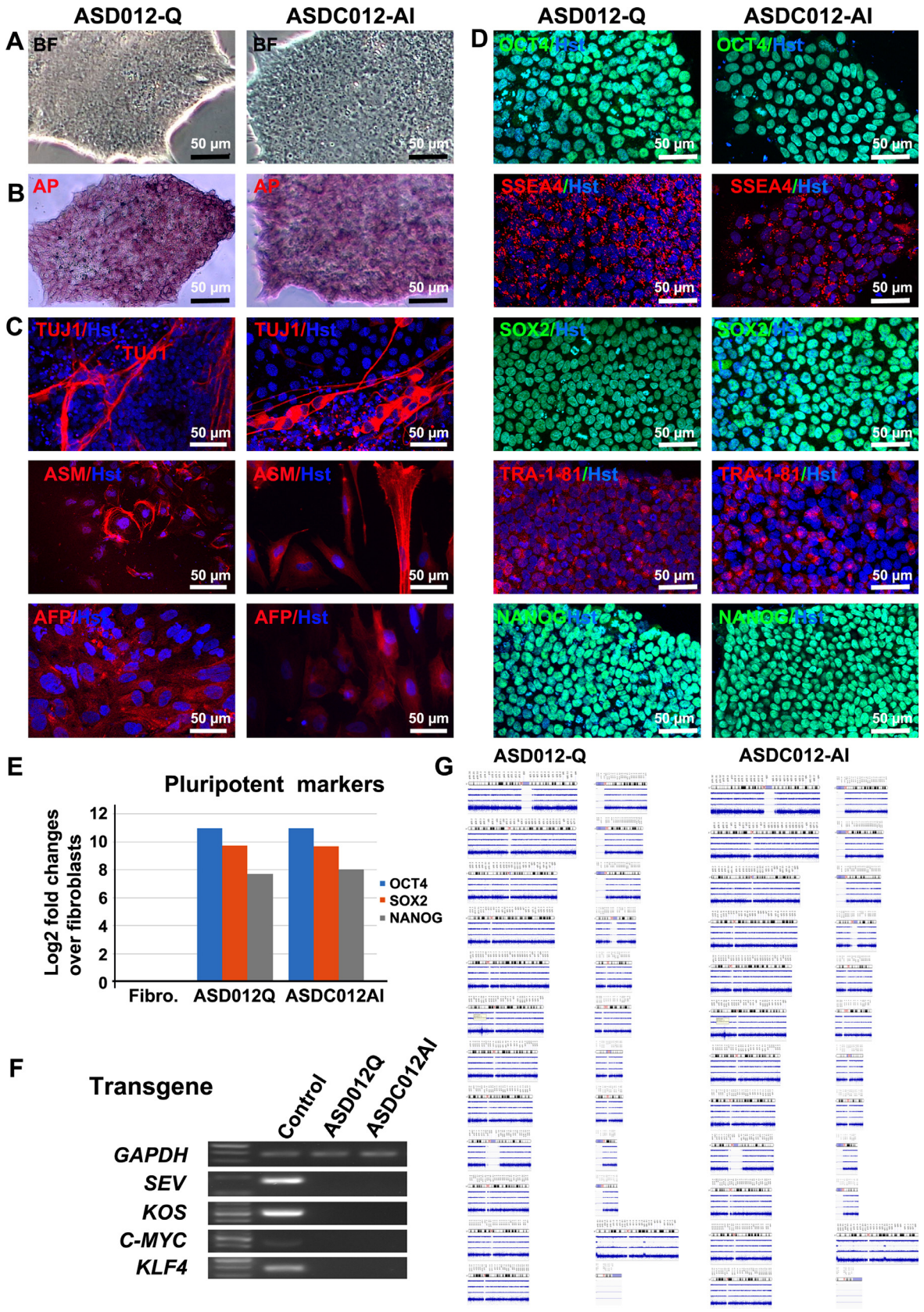

Fig. 1. Characterization of iPSC lines.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
NUIGi033-A	ASD012-Q	Male	6	Caucasian	Sporadic	ASD
NUIGi034-A	ASDC012-AI	Male	39	Caucasian	*N/A*	Parental control

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal morphology	Fig. 1A
Phenotype	Alkaline phosphatase staining	Positive staining	Fig. 1B
	Immunocytochemistry	Positive for OCT4, SSEA4, SOX2, TRA-1-81 and NANOG.	Fig. 1D
	qRT-PCR	Positive for SOX2, OCT4 and NANOG	Fig. 1E
	RT-PCR	Negative for Sendai vectors	Fig. 1F
Genotype	Single Nucleotide Polymorphism	No gross chromosomal alteration by reprogramming detected	Fig. 1G
Identity	Fingerprinting (STR analysis)	Tested 6 sites (D1S1656, D3S1358, D5S818, D7S796, D8S1179 and D10S1214), all matched	
Microbiology and virology	Mycoplasma	Detection by PCR, negative	Supplemental Fig. 1
Differentiation potential	Embryonic body formation	alpha-fetoprotein (AFP) for endoderm, alpha smooth muscle actin (α-SMA) for mesoderm, and beta-III tubulin (TUJ1) for ectoderm	Fig. 1C

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti-OCT4	1:1000	Cell Signaling Technology Cat# 2840, RRID:AB_2167691
Pluripotency markers	Mouse anti-SSEA4	1:500	Cell Signaling Technology Cat# 4755, RRID:AB_1264259
Pluripotency markers	Rabbit anti-SOX2	1:1000	Cell Signaling Technology Cat# 3579, RRID:AB_2195767
Pluripotency markers	Mouse anti-TRA-1-81	1:500	Cell Signaling Technology Cat# 2840, RRID:AB_2119060
Pluripotency markers	Rabbit anti-NANOG	1:1000	Cell Signaling Technology Cat# 3580, RRID: AB_2150399
Differentiation markers	Mouse anti-AFP	1:200	Sigma-Aldrich Cat# A8452, RRID:AB_258392
Differentiation markers	Mouse anti-TUJ1	1:500	Abcam Cat# ab78078, RRID:AB_2256751
Differentiation markers	Mouse anti-SMA	1:500	Cell Marque Corp Cat# 202M-96, RRID:AB_1157940
Secondary antibodies	AF488 Goat Anti-Rabbit IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904025
Secondary antibodies	AF555 Goat Anti-Mouse IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904022
Secondary antibodies	AF488 Goat Anti-Rabbit IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904025
Secondary antibodies	AF555 Goat Anti-Mouse IgG	1:1000	Cell Signaling Technology Cat# 4412, RRID:AB_1904022
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Sendai reprogramming vector (RT-PCR)	SeV/181 bp	For: GGATCACTAGGTGATATCGAGC Rev: ACCAGACAAGAGTTTAAGAGATATGTATC	
Sendai reprogramming vector (RT-PCR)	KOS (KLF4, OCT3/4, SOX2) /528 bp	For: ATGCACCGCTACGACGTGAGCGC Rev: ACCTTGACAATCCTGATGTGG	
Sendai reprogramming vector (RT-PCR)	KLF4/410 bp	For: TTCCTGCATGCCAGAGGAGCCC Rev: AATGTATCGAAGGTGCTCAA	
Sendai reprogramming vector (RT-PCR)	C-MYC/532 bp	For: TAACTGACTAGCAGGCTTGTCG Rev: TCCACATACAGTCCTGGATGATGATG	
Pluripotency markers (qPCR)	NANOG/149bp	For: ATAACCTTGGCTGCCGTCTC Rev: AGCCTCCCAATCCCAAACAA	
Pluripotency markers (qPCR)	OCT4/229bp	For: AACTTCACTGCACTGTACTCCTC Rev: CACCCTTTGTGTTCCCAATTCC	
Pluripotency markers (qPCR)	SOX2/187bp	For: AGACTTCACATGTCCCAGCACT Rev: CGGGTTTTCTCCATGCTGTTTC	
House-keeping genes (qPCR)	GAPDH/206bp	For: AGGGCTGCTTTTAACTCTGGT Rev: CCCCACTTGATTTTGGAGGGA	
STR analysis (PCR)	D1S1656	For: GTGTTGCTCAAGGGTCAACT Rev: GAGAAATAGAATCACTAGGGAACC	
STR analysis (PCR)	D3S1358	For: ACTGCAGTCCAATCTGGGT Rev: ATGAAATCAACAGAGGCTTGC	
STR analysis (PCR)	D5S818	For: GGGTGATTTTCCTCTTTGGT Rev: TTCCAATCATAGCCACA	
STR analysis (PCR)	D7S796	For: TTTTGGTATTGGCCATCCTA Rev: GAAAGGAACAGAGAGACAGGG	
STR analysis (PCR)	D8S1179	For: TTTTTGTATTTCATGTGTACATTCG Rev: TTTTTGTATTTCATGTGTACATTCG	
STR analysis (PCR)	D10S1214	For: TGCATAAAATATTGCCCCAAAAC Rev: TTGAAGACCAGTCTGGGAAG	

(Beijing, China). Axiom Analysis software (ThermoFisher, USA) was used to generate LogR ratio and B allele plots, using 83 samples to create an internal control. IGV software was used to visualize and test the molecular karyotyping of fibroblasts and derived iPSC lines.

Author declaration

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101722.

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