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Lab Resource: Multiple Cell Lines

Generation of two induced pluripotent stem cell (iPSC) lines from X-linked adrenoleukodystrophy (X-ALD) patients with adrenomyeloneuropathy (AMN)

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ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is an inherited disorder caused by a mutation in the ATP-binding cassette transporter subfamily D member 1 (ABCD1) gene. We generated two induced pluripotent stem cell (iPSC) lines from X-ALD patients with adrenomyeloneuropathy (AMN) by Sendai virus containing OCT4, SOX2, KLF4 and c-MYC. Established iPSC lines expressed various pluripotency markers, had differentiation potential of three germ layers in vitro, had normal karyotype and retained ABCD1 mutation.

Resource Table

Unique stem cell lines identifier	YUSEVi005-A YUSEVi006-A
Alternative names of stem cell lines	AMN 5 iPSC (YUSEVi005-A) AMN 6 iPSC (YUSEVi006-A)
Institution	[a] Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University [b] Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Epilepsy Research Institute
Contact information of distributor	Seungkwon You, bioseung@korea.ac.kr Hoon-Chul Kang, HIPO0207@yuhs.ac
Type of cell lines	iPSC
Origin	Human
Cell source	YUSEVi005-A: fibroblast YUSEVi006-A: fibroblast
Method of reprogramming	Sendai virus
Multiline rationale	Same disease non-isogenic cell lines
Gene modification	NO
Type of modification	N/A
Associated disease	X-linked adrenoleukodystrophy (X-ALD)
Gene/locus	ABCD1 gene/Xq28
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	2016.09.08/2016.09.22 (YUSEVi005-A) 2016.07.29/2016.08.05 (YUSEVi006-A)
Cell line repository/bank	N/A
Ethical approval	Ethical committee: Yonsei University Health System, Severance Hospital, Institutional Review Board Approval number: 4–2016-0194

Resource utility

These iPSC lines (YUSEVi005-A and YUSEVi006-A) will be useful for modeling X-ALD disease and developing drugs to treat this disease.

Resource details

X-linked adrenoleukodystrophy (X-ALD) is an inherited disorder caused by ATP-binding cassette transporter subfamily D member 1 (ABCD1) gene mutation (Mosser et al., 1993). Two human fibroblast cells from X-ALD patients with ABCD1 mutation were reprogrammed into iPSCs by Sendai virus containing OCT4, SOX2, KLF4, and c-MYC (Fig. 1A, Table 1). The established iPSC lines (YUSEVi005-A and YUSEVi006-A) expressed various pluripotency markers including OCT4, NANOG, and TRA-1-81 (Fig. 1B–C). One patient harboured one allele transition (G > A) of ABCD1 gene, which substituted Serine for Glycine at codon 512, as verified by genomic DNA sequencing of ABCD1 in YUSEVi005-A. The other patient harboured a deletion of three nucleotides of ABCD1 gene at codon 657 as verified by genomic DNA sequencing of ABCD1 in YUSEVi006-A (Fig. 1D). YUSEVi005-A and YUSEVi006-A could differentiate into cells of the three embryonic germ layers in vitro (Fig. 1E), had a normal karyotype without abnormalities in the number or structure of chromosomes (Fig. 1F), and were negative for *Mycoplasma* contamination (Fig. 1G). STR analysis showed that parental

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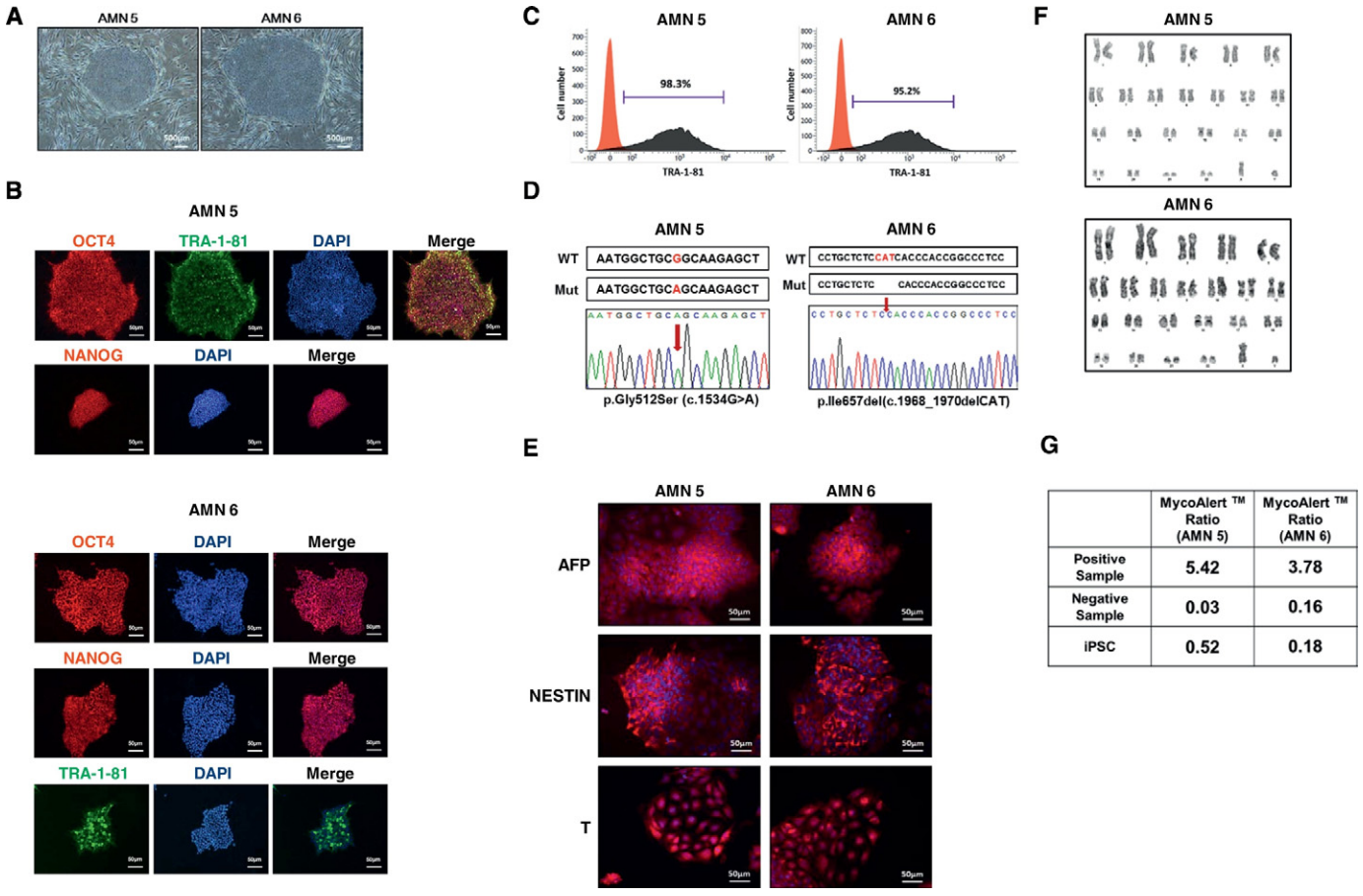

	MycoAlert ™ Ratio (AMN 5)	MycoAlert ™ Ratio (AMN 6)
Positive Sample	5.42	3.78
Negative Sample	0.03	0.16
iPSC	0.52	0.18

Fig. 1. Characterization of iPSC lines (YUSEVi005-A and YUSEVi006-A).

fibroblasts and the newly created YUSEVi005-A and YUSEVi006-A iPSC lines shared alleles with 100% match (Supplementary data, Table 2).

Materials and methods

Cell culture

Human fibroblasts were isolated from patients carrying a ABCD1 mutation and cultured in growth media (GM; DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 2 nM L-glutamine) at 37 °C in 5% CO_2.

Generation of iPSC from X-ALD patient fibroblasts

X-ALD patient fibroblasts were reprogrammed to iPSC using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Invitrogen) according to the manufacturer's instructions. After transduction, cells were reseeded on mouse embryonic fibroblast (STO) feeder cells (ATCC CRL-1503) and cultured in conventional human embryonic stem cell medium (Jang et al., 2011) for 30 days.

Immunocytochemistry

The iPSC lines (YUSEVi005-A and YUSEVi006-A) were fixed in 4% paraformaldehyde, incubated with primary antibodies overnight at 4 °C, and then incubated with secondary antibodies for 1 h at room temperature. Nuclei were stained with DAPI for 5 min at room temperature. Immunofluorescence was visualized under fluorescence microscope (Olympus IX71) (Table 3).

Flow cytometry analysis

The iPSC lines (YUSEVi005-A and YUSEVi006-A) were dissociated with accutase, blocked with 10% FBS for 30 min at 4 °C, incubated with a primary antibody for 1 h at 4 °C and then incubated with a secondary antibody for 1 h at 4 °C. Expression of surface marker of pluripotency was analysed by FACSVerse flow cytometer (BD

Table 1
Summary of lines

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
YUSEVi005-A	AMN 5	Male	34	Korean	p.Gly512Ser (c.1534G > A)	X-ALD
YUSEVi006-A	AMN 6	Male	43	Korean	p.Ile657del (c.1968_1970delCAT)	X-ALD

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Assess staining/expression of pluripotency markers: OCT4, NANOG, TRA-1-81	Fig. 1 panel B
	Flow cytometry	Assess antigen levels & cell surface markers: TRA-1-81: 98.3% (YUSEVi005-A) TRA-1-81: 95.2% (YUSEVi006-A)	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46 XY, Resolution 450 (YUSEVi005-A) 46 XY, Resolution 475 (YUSEVi006-A)	Fig. 1 panel F
Identity	Microsatellite PCR (mPCR)	N/A	
	STR analysis	18 locus tested. 100% match	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Hemizygote mutation	Fig. 1 panel D
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Fig. 1 panel G
Differentiation potential	In vitro differentiation	NESTIN, Brachyury (T), and α-feto protein	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

Biosciences). Negative samples were only labelled with the secondary antibody (Table 3).

In vitro differentiation

The iPSC lines (YUSEVi005-A and YUSEVi006-A) were cultured in chemically defined reprogramming medium (Chen et al., 2011) without basic fibroblast growth factor and transforming growth factor β for 3 days and then in the specified differentiation medium for 10 days. For ectodermal differentiation, cells were cultured in DMEM/F12 (Lonza) supplemented with 1× N2 (Thermo Fisher Scientific), 1× B27 (Thermo Fisher Scientific), 10 ng/ml of leukemia inhibitory factor (Millipore), 2 μM SB431542 and 3 μM CHIR99021. For mesodermal differentiation, cells were cultured in Advanced-RPMI (Thermo Fisher Scientific) supplemented with 8 μM CHIR99021. For endodermal differentiation, cells were cultured in DMEM-low glucose (Hyclone) supplemented with 10% FBS (Hyclone). The in vitro differentiation potential of the iPSC lines was confirmed by immunocytochemistry (Table 3).

Sequencing analysis of the ABCD1 mutant alleles and karyotyping

Genomic DNA was isolated from the iPSC lines using a Wizard® Genomic DNA Purification Kit (Promega). Mutation sequencing of AMN5 and AMN6 were performed using AMN5 and AMN6-specific primers (Table 3). Karyotyping was performed by GTG banding by Samkwang Medical Laboratories.

Mycoplasma contamination detection

The absence of mycoplasma contamination was confirmed using MycoAlert™ PLUS Mycoplasma Detection kit (Lonza).

STR analysis

Parent fibroblasts and their established iPSC lines (YUSEVi005-A and YUSEVi006-A) were authenticated using STR analysis by Cosmo Genetech.

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency markers	Rabbit anti-OCT4	1:200	Millipore Cat# AB3209, RRID: AB_2167706
Pluripotency markers	Goat anti-NANOG	1:200	R and D Systems Cat# AF1997, RRID:AB_355097
Pluripotency markers	Mouse anti-TRA-1-81	1:200	Millipore Cat# MAB4381, RRID:AB_177638
Differentiation markers	Mouse anti-NESTIN	1:200	Millipore Cat# MAB5326, RRID:AB_2251134
Differentiation markers	Rabbit anti-Brachyury	1:200	Abcam Cat# ab20680, RRID:AB_727024
Differentiation Markers	Goat anti-AFP	1:50	Santa Cruz Biotechnology Cat# sc-8108, RRID:AB_633815
Secondary antibodies	Alexa Fluor 488-conjugated Donkey Anti-Mouse IgM	1:500	Thermo Fisher Scientific Cat# A-21042, RRID:AB_2535711
Secondary antibodies	Cy3-conjugated Donkey Anti-Mouse IgG	1:500	Jackson ImmunoResearch Labs Cat# 715-165-151, RRID:AB_2315777
Secondary antibodies	Cy3-conjugated Donkey Anti-Goat IgG	1:500	Jackson ImmunoResearch Labs Cat# 705–165-147, RRID: AB_2307351
Secondary antibodies	Cy3-conjugated Donkey Anti-Rabbit IgG	1:500	Jackson ImmunoResearch Labs Cat# 711-165-152, RRID:AB_2307443
Primers			
	Target		Forward/Reverse primer (5′-3′)
Targeted mutation analysis/sequencing (YUSEVi005-A)	ABCD1(742 bp; NG_009022.2: 19921 to 20662)		CTGTGGCAGAATAGGCCCTT/CTCCCCCAAGATACTCTGCG
Targeted mutation analysis/sequencing (YUSEVi006-A)	ABCD1(NG_009022.2: 20649 to 23932; 3284 bp)		GTATCTTGGGGGAGGCAGAG/GGTGCTGCTGTCTCCTTCAT
Targeted mutation analysis/sequencing (YUSEVi006-A)	ABCD1(NG_009022.2: 23245 to 23615; 371 bp)		AAGGGGAAGTAGCAGCTGTG/AGGAGAGGGACAGGGTCAG

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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.2017.10.003.

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