Stem Cell Research 25 (2017) 266–269

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

Generation and characterization of human induced pluripotent stem cell (hiPSC) lines from an Alzheimer's disease (ASUi003-A) and non-demented control (ASUi004-A) patient homozygous for the Apolipoprotein e4 (APOE4) risk variant

Nicholas Brookhouser [a], Ping Zhang [b], Richard Caselli [c], Jean J. Kim [b], David A. Brafman [a,*]

[a] School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ 85287, USA
[b] Molecular and Cellular Biology, Stem Cells and Regenerative Medicine Center, Baylor College of Medicine, Houston, TX 77030, USA
[c] Department of Neurology, Mayo Clinic College of Medicine, Scottsdale, AZ 85259, USA

ARTICLE INFO

Article history:
Received 6 July 2017
Accepted 7 July 2017
Available online 10 July 2017

ABSTRACT

Although the majority of late-onset Alzheimer's disease (AD) patients are labeled sporadic, multiple genetic risk variants have been identified, the most powerful and prevalent of which is the e4 variant of the Apolipoprotein E (APOE) gene. Here, we generated human induced pluripotent stem cell (hiPSC) lines from the peripheral blood mononuclear cells (PBMCs) of a clinically diagnosed AD patient [ASUi003-A] and a non-demented control (NDC) patient [ASUi004-A] homozygous for the APOE4 risk allele. These hiPSCs maintained their original genotype, expressed pluripotency markers, exhibited a normal karyotype, and retained the ability to differentiate into cells representative of the three germ layers.

Resource table

Unique stem cell lines identifier	ASUi003-A ASUi004-A
Alternative names of stem cell lines	ASU-188 ASU-414
Institution	Arizona State University
Contact information of distributor	David Brafman, David.Brafman@asu.edu
Type of cell lines	iPSC
Origin	Human
Cell Source	Human peripheral blood mononuclear cells (PBMCs)
Method of reprogramming	CytoTune®-iPS 2.0 Reprogramming System
Multiline rationale	Homozygous for APOE 4 risk factor
Gene modification	No
Type of modification	N/A
Associated disease	Alzheimer's disease
Gene/locus	Apolipoprotein E (APOE)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	February, 2017
Cell line repository/bank	Not applicable
Ethical approval	Mayo Clinic Institutional Review Board; IRB # 15-008674

Resource utility

Polymorphisms in the Apolipoprotein (APOE) gene have been identified as the most prevalent of the risk factors associated with sporadic Alzheimer's disease (AD). As such, hiPSCs with various APOE genotypes will provide a valuable resource to study the mechanisms by which this risk factor contributes to AD onset and progression.

Resource details

Genome-wide association studies (GWAS) studies have identified several risk factors associated with increased probability of sporadic Alzheimer's disease (SAD) onset (Bettens et al., 2010). Of these risk factors, polymorphism in the Apolipoprotein E (APOE) gene, a lipoprotein transporter involved in cholesterol metabolism, is the strongest and most prevalent (Hauser and Ryan, 2013). Compared to individuals with an APOE e3/3 genotype (referred to as the 'risk neutral' allele), heterozygosity for the e4 allele increases AD risk by 3 fold, and homozygosity for the e4 allele increases risk up to 12 fold (Wolf et al., 2013). In this study, we report the generation of hiPSCs from two individuals from the Arizona APOE Cohort (for which recruitment and enrolment strategies have been described previously (Caselli et al., 2011)) that are homozygous for the APOE e4 allele— a clinically diagnosed AD patient (ASUi003-A, Mini-Mental Status Exam [MMSE] score = 14) who fulfilled published diagnostic criteria (McKhann et

* Corresponding author.
E-mail address: David.Brafman@asu.edu (D.A. Brafman).

http://dx.doi.org/10.1016/j.scr.2017.07.003

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
ASUi003-A	003	F	70	N/A	APOE: 112R/158R	Alzheimer's disease
ASUi004-A	004	M	65	N/A	APOE: 112R/158R	Healthy/non-demented

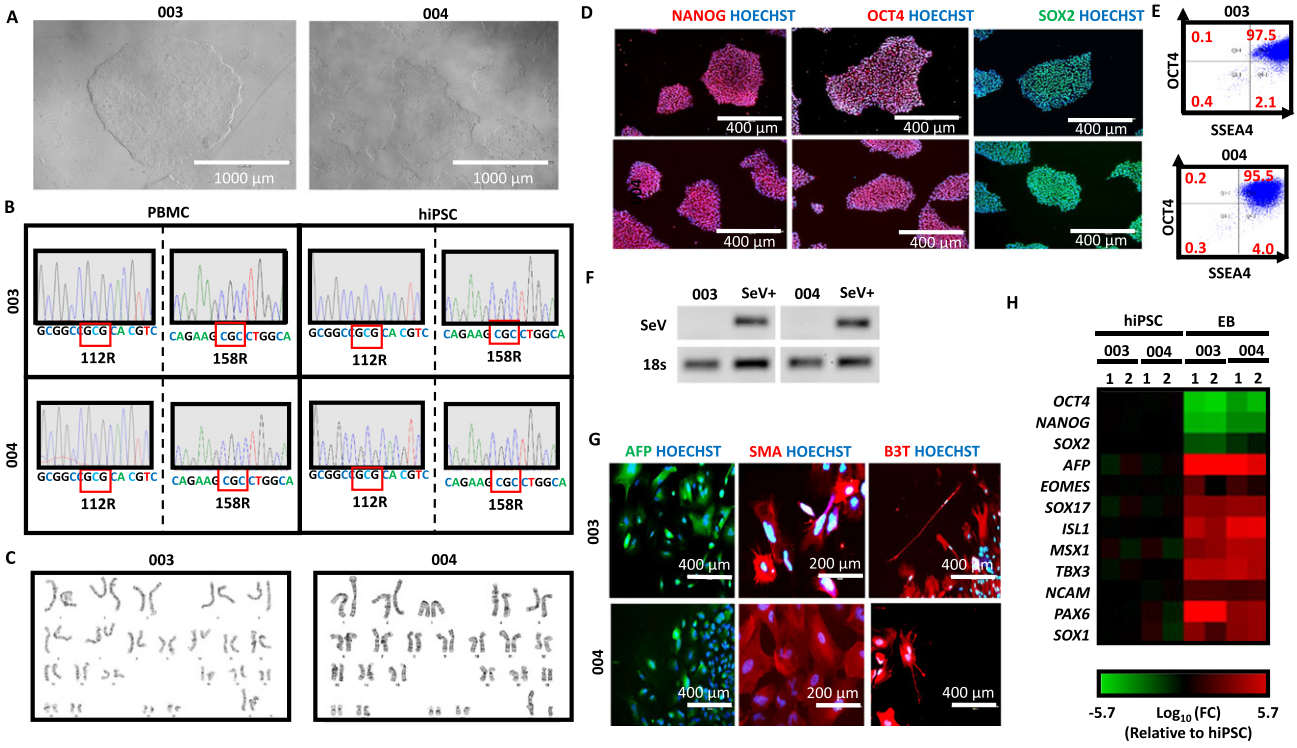

Fig. 1. Characterization of ASUi003-A and ASUi004-A human induced pluripotent stem cells (hiPSCs).

al., 2011) and an age-matched non-demented control patient (NDC; ASUi004-A, MMSE score = 30) (Table 1).

Peripheral blood mononuclear cells (PBMCs) were reprogrammed into hiPSCs using the non-integrating CytoTune®-iPS 2.0 Reprogramming System (Thermo Fisher Scientific). Several clones from each patient were isolated, expanded and characterized (Fig. 1 and Table 2). The expanded hiPSC clones displayed a typical pluripotent stem cell morphology (Fig. 1A). All expanded clones were confirmed to be negative for mycoplasma (Supplementary Table 1). Sequencing analysis of the hiPSCs at the APOE gene in exon 4 confirm homozygosity at the e4 allele, identical to the parental PBMCs [Fig. 1B; Note: Human APOE has three major isoforms, ApoE2, ApoE3, and ApoE4, which differ by two amino acid substitutions at residues 112 and 158 in exon 4—ApoE2 (Cys112, Cys158), ApoE3 (Cys112, Arg158), ApoE4 (Arg112, Arg158)]. Expanded clones

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1A
Phenotype	Immunocytochemistry	Positive staining for OCT4, NANOG, and SOX2	Fig. 1D
	Flow cytometry	OCT4/SSEA-4 Double Positive >95%	Fig. 1E
Genotype	Karyotype (G-banding) and resolution	46XX (ASUi003-A) 46XY (ASUi004-A) Resolution 450–550	Fig. 1C
Identity	Microsatellite PCR (mPCR)	Not performed	
	STR analysis	16 Loci All matched	Submitted in archive with journal
Mutation analysis	Sequencing	Homozygous for Apolipoprotein e4 risk variant	Fig. 1B
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence Negative	Supplementary Table 1
Differentiation potential	Embryoid body	Endoderm (AFP, EOMES, SOX17), mesoderm (ISL1, MSX1, SMA, TBX3), and ectoderm (B3T, NCAM, PAX6, SOX1)	Fig. 1G and H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	
	HLA tissue typing	Not performed	

maintained a normal euploid karyotype (Fig. 1C). Immunofluorescent staining (Fig. 1D) and flow cytometry (Fig. 1E) revealed that the hiPSCs expressed high levels of pluripotency-associated markers NANOG, OCT4, SOX2, and SSEA-4. Absence of viral transgenes in expanded clones was confirmed by RT-PCR (Fig. 1F). Finally, to verify pluripotency, hiPSCs were spontaneously differentiated *in vitro* through embryoid body (EB) formation. Immunofluorescence (Fig. 1G) and gene expression analysis (Fig. 1H) of EBs revealed downregulation of pluripotency-associated markers (OCT4, NANOG, SOX2) and upregulation of genes associated with endoderm (AFP, EOMES, SOX17), mesoderm (ISL1, MSX1, SMA, TBX3), and ectoderm (B3T, NCAM, PAX6, SOX1).

Materials and methods

Reprogramming of PBMCs

Peripheral blood samples were collected in BD Vacutainer cell preparation tubes and centrifuged for 30 min at 1800 RCF. Isolated PBMCs were cultured in expansion medium (EM; QBSF-60 [Fisher Scientific]

Table 3
Reagent details.

Antibodies used for immunocytochemistry and flow cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT4	1:50	Santa Cruz, Cat# sc-5279 RRID: 628051
	Mouse anti-NANOG	1:50	Santa Cruz, Cat# sc-293121 RRID: 10548762
	Goat anti-SOX2	1:50	Santa Cruz, Cat# sc-17320 RRID: 2286684
	Mouse IgG1 anti-OCT4-PE	1:10	BD Biosciences, Cat# 560186 RRID: 1645331
	Mouse IgG3 anti-SSEA4-APC	1:10	R&D Systems, Cat# FAB1435A RRID: 494994
Differentiation Markers	Rabbit anti-AFP	1:50	Santa Cruz, Cat# sc-15375 RRID: 2223935
	Mouse-anti SMA	1:50	Santa Cruz, Cat# sc-53015 RRID: 628683
	Mouse anti-B3T	1:100	Fitzgerald, Cat# 10R-T136A RRID: 1289248
Secondary Antibodies	Alexa 488 Donkey anti-goat IgG	1:200	Thermo Fischer, Cat# A11055 RRID: 2534102
	Alexa 647 Donkey anti-mouse IgG	1:200	Thermo Fisher, Cat# A31571 RRID: 162542
	Alexa 488 Donkey anti-rabbit IgG	1:200	Thermo Fisher, Cat# A21206 RRID: 141708
Isotype Control	Mouse IgG1-PE	1:10	BD Biosciences, Cat# 559320 RRID: 397218
	Mouse IgG1-APC	1:10	R&D Systems, Cat# IC007A RRID: 952035

Primers		
	Target	Forward/Reverse primer (5′-3′)
SeV Transgene (RT-PCR)	*SeV*	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC
Pluripotency Markers (qPCR)	*NANOG*	CAATGGTGTGACGCAGGGAT GGACTGTTCCAGGCCTGATT
	OCT4	CAAAGCAGAAACCCTCGTGC CTCGGACCACATCCTTCTCG
	SOX2	GGATAAGTACACGCTGCCCG ATGTGCGCGTAACTGTCCAT
Germ Layer Markers (qPCR)	*AFP*	AGAGTTGCTAAAGGATACCAGGA AGGCCAATAGTTTGTCCTCAC
	EOMES	AAATGGGTGACCTGTGGCAAAGC CTCCTGTCTCATCCAGTGGGAA
	ISL1	GGATTTGGAATGGCATGCGG CATTTGATCCCGTACAACCTGA
	MSX1	CCCTGGTGCTGTACCCC GGTCCCTTCAACCTACCTT
	NCAM	AGACCCCATTCCCTCCATCA TGTGCCCATCCAGAGTCTTT
	SOX1	AATACTGGAGACGAACGCCG CCCTCGAGCAAAGAAAACGC
	SOX17	GAATCCAGACCTGCACAACG CTCTGCCTCCTCCACGAAG
	TBX3	ATTTCACAATTCTCGGTGGA TATAATTCCCCTGCCACGTA
	PAX6	CTTCGCTAATGGGCCAGTGA TCAGATTCCTATGCTGATTGGTGA
House-Keep Gene (qPCR)	*18s*	GTAACCCGTTGAACCCCATT CCATCCAATCGGTAGTAGCG
Genotyping (PCR)	*APOE*	TAAGCTTGGCACGGCTGTCCAAGGA ACAGAATTGGCCCCGGCCTGGTACAC

supplemented with 100 μg/mL Primocin [Fisher Scientific], 1% penicillin/streptomycin [Thermo Fisher], 50 μg/mL ascorbic acid [Sigma], 50 ng/mL SCF [R&D], 10 ng/mL IL-3 [R&D], 2 U/mL EPO [R&D], 40 ng/mL IGF-1 [R&D], 1 μM Dexamethasone [Sigma]). After 9–12 days of expansion, 2.5×10^5 PBMCs were resuspended in EM and transferred to a 12 well plate. Sendai viruses (SeV; CytoTune®-iPS 2.0 Reprogramming Kit [Thermo Fisher]) were added at a minimum multiplicity of infection (MOI) of 5. Three days after transduction, cells were cultured on hESC-qualified Matrigel® (Corning) in TeSR-E7 medium for 7 days, and then switched to TeSR-E8 (E8) medium (STEMCELL Technologies). After 21 days, individual hiPSC colonies were mechanically isolated and expanded. After mechanically passaging for the first 3 passages, hiPSCs were non-enzymatically passaged using ReLeSR™ (STEMCELL Technologies) and cryopreserved. For routine maintenance and downstream characterization experiments, hiPSCs were passaged every 4–5 days with Accutase (Thermo Fisher) and 5 μM Rho kinase inhibitor (Y-27632; Biogems). Mycoplasma testing was performed with the MycoAlert PLUS kit (Lonza) and the Lucetta™ Luminometer (Lonza).

Quantitative RT-PCR

RNA was isolated from cells (NucleoSpin RNA Kit, Clontech) and reverse transcription was performed (iScript RT Supermix, Bio-Rad). Quantitative PCR was performed with SYBR green dye on a Bio-Rad CFX384 TouchTM Real-Time PCR Detection System. Primer sequences are provided in Table 3. Gene expression was normalized to 18S rRNA levels. Relative fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

APOE genotyping

Genomic DNA was isolated from cells using the DNeasy kit (Qiagen). PCR of APOE exon 4 was performed using primers in Table 3. Sanger sequencing was performed on PCR products (ASU DNA Core Lab).

Karyotyping and STR analysis

Cytogenetic analysis was performed using standard protocols for G-banding (Baylor Miraca Genetics Laboratories). Short tandem repeat (STR) analysis was performed with Promega's PowerPlex® 16 multiplex STR system (Cell Line Genetics).

Flow cytometry

Cells were dissociated, fixed, and stained using standard procedures for flow cytometry. Cells were analysed on an LSR II flow cytometer (BD Biosciences). Antibodies are listed in Table 3.

Immunofluorescence

Cultures were fixed, permeabilized, and stained using standard procedures for immunofluorescence. Antibodies used are listed in Table 3. Nucleic acids were stained for DNA with Hoechst 33342 (2 μg/mL; Life Technologies). Imaging was performed using an EVOS microscope (Thermo Fisher).

In vitro embryoid body (EB) formation

HiPSCs were harvested using ReLeSR™ (StemCell Technologies) and plated on low attachment plates in E8 medium. The following day, the media was changed to differentiation medium (DM; DMEM/F12, 20% FBS, 1% Pen/Strep). After 5 days, EBs were plated on Matrigel-coated plates and cultured with DM. After 14 days, cells were fixed, permeabilized, and stained for germ layer markers. In addition, cells were dissociated using Accutase, RNA was isolated, and RT-PCR was performed to assess expression of pluripotency and germ layer markers.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.003.

Author disclosure statement

There authors declare no competing financial interests in this study.

Acknowledgments

This work was supported by an ASU-Mayo Seed Grant to D.A.B. and the Arizona Alzheimer's Consortium.

References

Bettens, K., Sleegers, K., Van Broeckhoven, C., 2010. Current status on Alzheimer disease molecular genetics: from past, to present, to future. Hum. Mol. Genet. 19, R4–R11.

Caselli, R.J., Dueck, A.C., Locke, D.E., Hoffman-Snyder, C.R., Woodruff, B.K., Rapcsak, S.Z., Reiman, E.M., 2011. Longitudinal modeling of frontal cognition in APOE epsilon4 homozygotes, heterozygotes, and noncarriers. Neurology 76, 1383–1388.

Hauser, P.S., Ryan, R.O., 2013. Impact of apolipoprotein E on Alzheimer's disease. Curr. Alzheimer Res. 10, 809–817.

McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack Jr., C.R., Kawas, C.H., Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rossor, M.N., Scheltens, P., Carrillo, M.C., Thies, B., Weintraub, S., Phelps, C.H., 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 7, 263–269.

Wolf, A.B., Valla, J., Bu, G., Kim, J., LaDu, M.J., Reiman, E.M., Caselli, R.J., 2013. Apolipoprotein E as a beta-amyloid-independent factor in Alzheimer's disease. Alzheimers Res. Ther. 5, 38.