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Generation of inducible pluripotent stem cell lines from Alzheimer's disease patients with *APOE* e3/e3 genotype

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Abstract

iPSCs were generated from B lymphocytes of two Alzheimer's disease (AD) patients homozygous for the *APOE* e3 allele. The iPSCs express pluripotency-specific markers and have the capacity to differentiate into all three embryonic germ layers. Karyotyping analyses confirmed the iPSCs have normal karyotypes. These iPSCs can be utilized as an in vitro model to study AD and to evaluate efficacies of new treatments.

Resource Table: Please fill in right-hand column of the table below. All information requested in the table is MANDATORY, except where otherwise indicated. Manuscripts with incomplete or incorrect information will be sent back to author

Unique stem cell lines identifier	1. Set up an account at https://hpscereg.eu/about/naming-tool 2. The system generates and guarantees a unique name based on: researcher's institution; type of cell line type (iPSC/hESC); additional clone from patient or subclone of a line already present in the database. 3. Include all the unique cell lines name generated HERE. <i>UABi001-A</i> <i>UABi002-A</i>
Alternative names of stem cell lines	<i>AG10039-iPSC-1</i> <i>AG08242-iPSC-1</i>
Institution	<i>University of Alabama at Birmingham</i>
Contact information of distributor	<i>Rui Zhao, ruizhao@uab.edu</i>
Type of cell lines	<i>iPSC</i>

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2021.102498>.

Origin	<i>Human</i>
Cell Source	<i>B lymphocytes</i>
Clonality	<i>Clonal</i>
Method of reprogramming	<i>Transfection of non-integrative episomal plasmids</i>
Multiline rationale	<i>same disease non-isogenic cell lines</i>
Gene modification	<i>No</i>
Type of modification	<i>N/A</i>
Associated disease	<i>Alzheimer's disease</i>
Gene/locus	<i>APOE e3/e3</i>
Method of modification	<i>N/A</i>
Name of transgene or resistance	<i>N/A</i>
Inducible/constitutive system	<i>N/A</i>
Date archived/stock date	<i>6.26.20</i>
Cell line repository/bank	<i>N/A</i>
Ethical approval	<i>Obtained from the NIA Aging Cell Repository, which is administered by the Coriell Institute for Medical Research - please see attached Executed Assurance Form for Cell lines and DNA samples</i>

1. Resource utility

The two iPSC lines, UABi001-A and UABi002-A, are derived from B lymphocytes of two Alzheimer's disease (AD) patients homozygous for the APOE e3 allele. These iPSCs will be used to study AD and to evaluate efficacies of new treatments.

2. Resource details

Alzheimer's disease (AD), manifested by a progressive decline of cognitive ability, is incurable. AD is caused by a complex interplay of genetic and environmental risk factors. Patient-derived iPSCs, which carry all genetic factors contributing to the disease and have the capacity to differentiate into disease-relevant cell types (e.g., neurons), have been a promising tool to study the disease. The APOE gene mediates lipid transport among various tissues and organs but has a variant allele, APOE e4, that has been identified as a major genetic risk factor of AD. Although APOE e4 increases the risk of AD by two to three-fold compared to the more prevalent APOE e3 allele (Wang et al., 2021), a significant number of AD patients are homozygous for the APOE e3 allele. In this study, iPSC lines were generated from two AD patients homozygous for the APOE e3 allele (Table 1), which provide a valuable resource to study APOE e4-independent AD. AD patient-derived B lymphocytes were acquired from the National Institute of Aging (NIA) Aging Cell Repository and transfected with the non-integrative episomal reprogramming plasmids expressing OCT4, SOX2, KLF4, L-MYC, LIN28 and TP53 shRNA Okita et al. (2011). Multiple iPSC colonies were observed five to six weeks after transfection. One iPSC clone randomly selected from each patient, designated as UABi001-A and UABi002-A, was fully characterized. Both UABi001-A and UABi002-A iPSCs form colonies with the morphology of normal human pluripotent stem cells and express pluripotent stem cell markers OCT4, SOX2, NANOG, and Tra-1–60 (Fig. 1A–D). Teratoma formation assays

confirmed that both UABi001-A and UABi002-A can differentiate into all three embryonic germ layers (Fig. 1E–F). Furthermore, karyotyping analyses demonstrated that UABi001-A has a normal 46, XX and UABi002-A has a normal 46, XY karyotype, respectively (Fig. 1G–H). iPSCs from UABi001-A and UABi002-A were verified to derive from the two AD patients by Short Tandem Repeat (STR) analysis (Supplementary file 1). Each iPSC line was confirmed to be homozygous for the APOE e3 allele by automatic sequencing (Supplementary Fig. 1A) and are free from mycoplasma contamination (Supplementary Fig. 1B). Together, our data demonstrate that both iPSC lines are fully reprogrammed and can be used to study AD and to evaluate efficacies of potential therapies (Table 2).

3. Materials and methods

3.1. Cell culture and reprogramming

B lymphocytes from two AD patients (AG10039 and AG08242) were acquired from NIA Aging Cell Repository administered by the Coriell Institute for Medical Research. B lymphocytes were cultured and maintained in D15 medium (DMEM containing 15% FBS). 1×10^6 cells were transfected with the episomal reprogramming plasmids pCXLE-hOCT4-shp53, pCXLE-hSOX2-hKLF4, pCXLE-hMYCL-hLIN28, and pCXLE-GFP (1 μ g of each plasmid per transfection) by nucleofection (Nucleofector 2b, program X005, Lonza). Cells were seeded onto tissue culture plates coated with hESC-qualified GelTrex Basement Membrane Matrix (Thermo Fisher) and cultured in D15 for seven days. Cells were then cultured in E7 medium (Chen et al., 2011), for 21 days before changing to E8 medium (Chen et al., 2011) until iPSC colonies were observed. iPSC colonies were picked, expanded, and maintained in mTeSR plus medium (StemCell Technologies).

3.2. Immunostaining

Immunostaining was performed as described (Liu and Zhao, 2016). In brief, cells were fixed in 4% formaldehyde overnight and then blocked in protein block (Agilent) for 30 min before incubating in the indicated primary antibody at 4 °C overnight. Cells were then incubated with the secondary antibody at room temperature for 45 min. Images were acquired by a Nikon Ti-S microscope and processed by the Photoshop CS6 software. See Table 3 for detailed antibody information.

3.3. Flow cytometry for cell surface marker

Flow cytometry analysis was performed as described (Liu and Zhao, 2016). iPSCs were dissociated by Accutase (Corning), incubated with the primary antibody against Tra-1–60 for 1 h, followed by the FITC conjugated secondary antibody for 45 min on ice. Samples were immediately analyzed by a BD Fortessa (BD Biosciences) flow cytometer and data were analyzed by Flowjo VX software (BD Biosciences).

3.4. Karyotyping and teratoma formation assay

UABi001-A and UABi002-A were submitted to Cell Line Genetics (Madison, WI) for G-band karyotyping, which analyzes twenty G-banded metaphase cells of each cell line. Teratoma formation assay was conducted as described (Liu et al., 2015). Briefly, 1×10^6 – 5×10^6 iPSCs were suspended into 100 μ l of DMEM/F12 medium containing

30% hESC-qualified GelTrex Basement Membrane Matrix (Thermo Fisher) and injected subcutaneously into six week-old NOD SCID gamma (NSG) mice (005557, The Jackson Laboratory). Tumors were harvested, fixed with 10% formalin, and submitted to the Comparative Pathology Laboratory at University of Alabama at Birmingham (UAB). All animal experiments were controlled under the guidelines from UAB and NIH.

3.5. STR analysis

Genomic DNA from patient B-lymphocytes and UABi001-A and UABi002-A iPSCs were prepared by Quick-gDNA miniprep kit (Zymo) and submitted to the Genomics Core Laboratories at the UAB Hefflin Center for Genomic Sciences. The STR analysis was performed using the GenePrint 24 Kit (Promega) and an ABI 3730xl Genetic Analyzer (Thermo Fisher).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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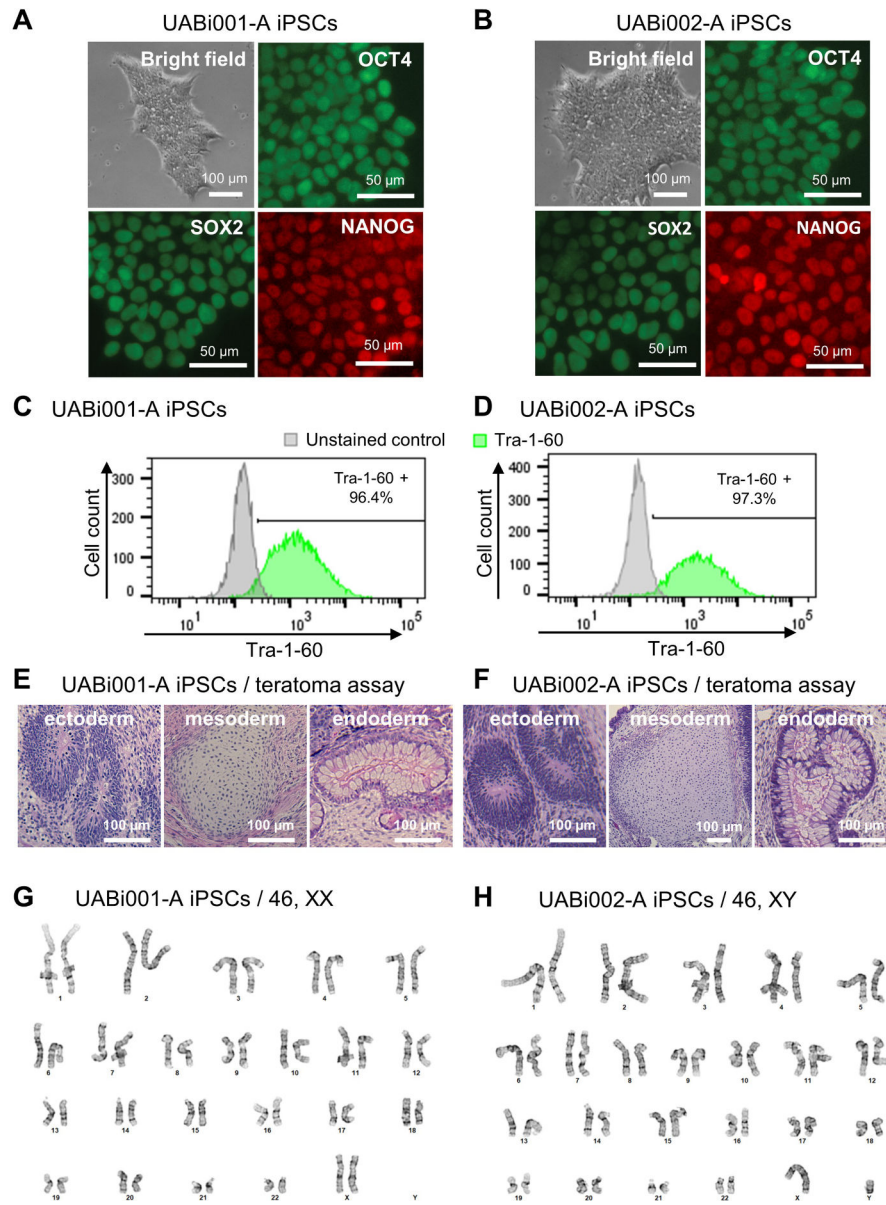


Fig. 1. Characterization of the Alzheimer's disease patient-derived iPSCs.

Table 1

Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UABi001-A	UABi001-A	Female	39	Caucasian	APOE e3/e3	Alzheimer's Disease
UABi002-A	UABi002-A	Male	72	Caucasian	APOE e3/e3	Alzheimer's Disease

Table 2

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Figure 1 panel A & B
Phenotype	Qualitative analysis (immunofluorescence)	Assess staining/expression of pluripotency markers: Oct4, Nanog, Sox2	Figure 1 panel A & B
	Quantitative analysis (Flow cytometry)	Assess % of positive cells or transcripts for antigen & cell surface markers: > 95% Tra 1-60	Figure 1 panel C & D
Genotype	Karyotype (G-banding) and resolution [mandatory]	UABi001-A, 46XX; UABi002-A, 46XY; Detect aberrations > 5 Mb	e.g Figure 1 panel G & H
Identity	Microsatellite PCR (mPCR) OR STR analysis [mandatory]	STR performed for UABi001-A and UABi002-A 24 different STR markers were tested and both iPSC lines matched with the donor B-lymphocytes	e.g. supplementary file 2 e.g. submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	APOE homozygous E3/E3	Supplementary Fig. 1A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Supplementary Fig. 1B
Differentiation potential	Teratoma formation	proof of three germlayers formation	Figure 1 panel E and F
Donor screening (OPTIONAL)	N/A	N/A	N/A
Genotype additional info (OPTIONAL)	N/A	N/A	N/A
	N/A	N/A	N/A

Table 3

Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-SOX2 IgG	1:100	BD Biosciences Cat# BD561469; RRID: AB_10694256
	Mouse anti-OCT4 IgG	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051
	Mouse anti-Tra-1-60 IgM	1:100	Santa Cruz Biotechnology Cat# sc-21705, RRID: AB_628385
	Goat anti-NANOG	1:100	R and D Systems Cat# AF1997, RRID:AB_355097
Differentiation Markers			
Secondary antibodies	Alexa 488 goat anti-mouse IgG	1:300	Molecular Probes Cat# A-11029, RRID:AB_138404
	Alexa 488 goat anti-mouse IgM	1:300	Molecular Probes Cat# A-21042, RRID:AB_141357
	Alexa 568 donkey anti-goat IgG	1:300	Molecular Probes Cat# A-11057, RRID:AB_142581
Primers			
	Target	Forward/Reverse primer (5'-3')	
Genotyping	APOE	GTCTCCTTCTCTCGGCCTCT/GCGCTTCTGCAGGTCATC	