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Lab resource: Stem Cell Line

Human induced pluripotent stem cells generated from a patient with idiopathic basal ganglia calcification

Yuichiro Yada [a,b], Takayuki Kondo [a,b,c], Mika Suga [a,b], Kayoko Tsukita [a,b], Takako Enami [b,c], Ran Shibukawa [a], Yukako Sagara [a], Yasue Okanishi [a], Keiko Imamura [a,b,c], Takeshi Kihara [d], Haruhisa Inoue [a,b,c,*]

[a] iPSC-based Drug-Discovery and Development Team, RIKEN BioResource Research Center, Kyoto, Japan
[b] Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan
[c] Medical-risk Avoidance based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project (AIP), Kyoto, Japan
[d] Kobe City Medical Center West Hospital, 2-4 Ichibancho, Nagata-ku, Kobe, Japan

A B S T R A C T

Idiopathic basal ganglia calcification (IBGC) is a rare neurodegenerative disease, characterized by abnormal calcium deposits in basal ganglia of the brain. The affected individuals exhibit movement disorders, and progressive deterioration of cognitive and psychiatric ability. The genetic cause of the disease is mutation in one of several different genes, SLC20A2, PDGFB, PDGFRB, XPR1 or MYORG, which inheritably or sporadically occurs. Here we generated an induced pluripotent stem cell (iPSC) line from an IBGC patient, which is likely be a powerful tool for revealing the pathomechanisms and exploring potential therapeutic candidates of IBGC.

1. Resource table

Unique stem cell line identifier	BRCi007-A
Alternative name of stem cell line	HPS3900
Institution	RIKEN BioResource Research Center (BRC), Kyoto, Japan
Contact information of distributor	Haruhisa Inoue haruhisa.inoue@riken.jp
Type of cell line	iPSC
Origin	Human
Additional origin information	Age: 80 Sex: male Ethnicity: Japanese
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Episomal vectors (Oct3/4, mp53DD, Sox2, KLF4, L-MYC, LIN28, EBNA1)
Genetic Modification	No
Type of Modification	Not available
Associated disease	Idiopathic basal ganglia calcification (IBGC)
Gene/locus	Not identified
Method of modification	Not available
Name of transgene or resistance	Not available
Inducible/constitutive system	Not available
Date archived/stock date	June 2018
Cell line repository/bank	RIKEN BioResource Research Center, Japan https://web.brc.riken.jp/en/
Ethical approval	Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University (approved No. = R0091, G259) Ethics Committee of the RIKEN BioResource Research Center (approved No. Tsukuba 29–1)

2. Resource utility

Abnormal calcification occurs in the brains of patients with in idiopathic basal ganglia calcification (IBGC). Development of human induced pluripotent stem cells (iPSCs) may offer a cell model for investigating IBGC and potential therapeutic approaches.

3. Resource details

IBGC, also referred to as primary familial brain calcification (PFBC), is characterized by abnormal deposits of calcium or hydroxyapatite typically in basal ganglia of the brain (Wang et al., 2012). The symptoms

* Corresponding author at: iPSC-based Drug-Discovery and Development Team, RIKEN BioResource Research Center, Kyoto, Japan.
E-mail address: haruhisa.inoue@riken.jp (H. Inoue).

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of the disease are movement disorders such as parkinsonism, which include slow movement (bradykinesia), muscle rigidity and tremors, and neuropsychiatric symptoms including dementia and psychosis. The disease is progressive and the symptoms worsen over time. Fundamental treatment for IBGC has not been established. The frequency of IBGC is fairly rare, although the expected number of patients is considerably more than the currently detected cases because detection of calcium deposit in the brain requires brain imaging. The genetic cause of IBGC is mutation in one of several disease-associated genes, which include SLC20A2, PDGFB, PDGFRB, XPR1 or MYORG (Yao et al., 2018). Of note is that some carriers remain asymptomatic (Nicolas et al., 2015). The mutation occurs both inheritably and sporadically. However, the molecular mechanism and pathology of IBGC are still unclear. In this report, human iPSCs were generated from peripheral blood cells of an IBGC patient for use as a substrate in modelling studies and for investigating the pathomechanisms of the disease (Table 1). Episomal vectors were used to transduce reprogramming factors (Okita et al., 2013) into peripheral blood mononuclear cells (PBMCs) from an IBGC patient, and IBGC-iPSCs that expressed the pluripotency markers NANOG, OCT3/4, and TRA1-60 were established (Fig. 1A). Flow cytometric analysis showed that 99.9% of the iPSCs were positive for SSEA-4 and 91.6% were positive for TRA1-60 (Fig. 1B). The differentiation capacity of the cell line was demonstrated by its ability to differentiate the three germ layers in vitro; immunostaining confirmed the presence of the mesodermal marker smooth muscle actin (SMA), the endodermal markers SOX17 and α-Fetoprotein (AFP), and the ectodermal marker βIII-Tubulin (Fig. 1C). The cell line maintained a normal karyotype after the reprogramming process (Fig. 1D).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal, human ESC-like morphology	Fig. 1 panel A
Phenotype	Immunocytochemistry	Assess staining of pluripotency markers: NANOG, Oct3/4, TRA1-60	Fig. 1 panel A
	Flow cytometry	SSEA-4 99.9% TRA1-60 91.6%	Fig. 1 panel B
Genotype	Karyotype (G-banding)	46XY Resolution 400 bands	Fig. 1 panel D
Identity	Microsatellite PCR (mPCR)	Not performed	Not performed
	STR analysis	16 loci, matched	Not shown but available with the authors
Mutation analysis	Sequencing	Not performed	Not performed
	Southern Blot OR WGS	Not performed	Not performed
Microbiology and virology	Mycoplasma	Mycoplasma testing by Vero-Hoechst method and nested-PCR. Negative	Not shown but available with the authors
Differentiation potential	Embryoid body formation OR Teratoma formation OR Scorecard	Describe expression of genes in embryonic bodies: SOX-17, AFP, αSMA, and βIII-Tubulin.	Fig. 1 panel C
Donor screening (OPTIONAL)	HIV, HTLV-1, Hepatitis B, Hepatitis C	Negative	Not shown but available with the authors
Genotype additional info	Blood group genotyping	Not available	Not available
	HLA tissue typing	Not available	Not available

4. Materials and methods

4.1. Ethics statement

The generation and use of human iPSCs were approved by Ethics Committees of RIKEN BioResource Research Center, and the Department of Medicine and Graduate School of Medicine, Kyoto University. Formal informed consent was obtained from the patient. All experiments in this study were performed in accordance with the approved guidelines.

4.2. Establishment of iPSCs

Human complementary DNAs of reprogramming factors were transduced into peripheral blood mononuclear cells (PBMCs) using episomal vectors: pCE-hOCT3/4 (OCT3/4, 1.05 μg); pCE-hSK (SOX2 and KLF4, 1.05 μg); pCE-hUL (L-MYC and LIN28, 1.05 μg); pCE-mp53DD (p53 carboxy-terminal dominant-negative fragment, 1.05 μg); and pCXB-EBNA1 (EBNA1, 0.8 μg). The vectors were transduced by using a 4D-Nucleofector X-unit with the program EO-117 (Lonza, Basel, Switzerland). In-house manufactured vectors were used in the reprogramming; these are also available from addgene (https://www.addgene.org/). After transduction, the generated iPSCs were cultured under feeder-free conditions (Nakagawa et al., 2014); the iPSCs were seeded onto laminin 511 E8 coated plates and maintained in StemFit medium (AK02N, Ajinomoto, Tokyo, Japan). Single cell isolates were passaged every 6–8 days with TrypLE Select (Thermo Fisher Scientific, Waltham, MA) diluted 1:1 with 0.5 mM EDTA solution. After the passaging, 10 μM Y-27632 (Nacalai Tesque, Kyoto, Japan) was added to the culture medium for one day and was then removed by replacing with the fresh medium on the next day. The cultures were maintained in a 5% CO_2 incubator at 37 °C.

4.3. In vitro differentiation into the three germ layer cell types

For differentiation of the three germ layer cell types, the generated iPSCs were collected by TrypLE select (Thermo Fisher Scientific) and transferred to a V-bottom 96-well plate (Sumitomo Bakelite, Tokyo, Japan). The cells were cultured in DMEM/F12 with GlutaMAX (Thermo Fisher Scientific) supplemented with 20% Knockout Serum Replacement (KSR; Thermo Fisher Scientific), 1 mM Non-Essential Amid Acid (NEAA; Thermo Fisher Scientific), penicillin/streptomycin (PS; Thermo Fisher Scientific) and 10 μM Y-27632 (Nacalai Tesque, Kyoto, Japan); embryonic bodies (EBs) were generated in culture. On day 8, EBs were collected and moved to a Matrigel-coated plate. The EBs were induced to differentiate into the three germ layers by culturing in DMEM/F12 with GlutaMAX containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific) for an additional 7 days.

4.4. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde/PBS and then incubated in blocking buffer (5% Blocking One/PBS, Nacalai Tesque) with 0.2% Triton X-100 (Nacalai Tesque)/PBS. The cells were kept overnight in blocking buffer containing primary antibodies and subsequently secondary antibodies. The antibodies used in the experiments are listed in Table 2. Cell nuclei were stained with DAPI (Thermo Fisher Scientific). Phase contrast and fluorescence images were obtained using a BZ-X710 microscope (Keyence, Osaka, Japan). Scale bars indicate 100 μm.

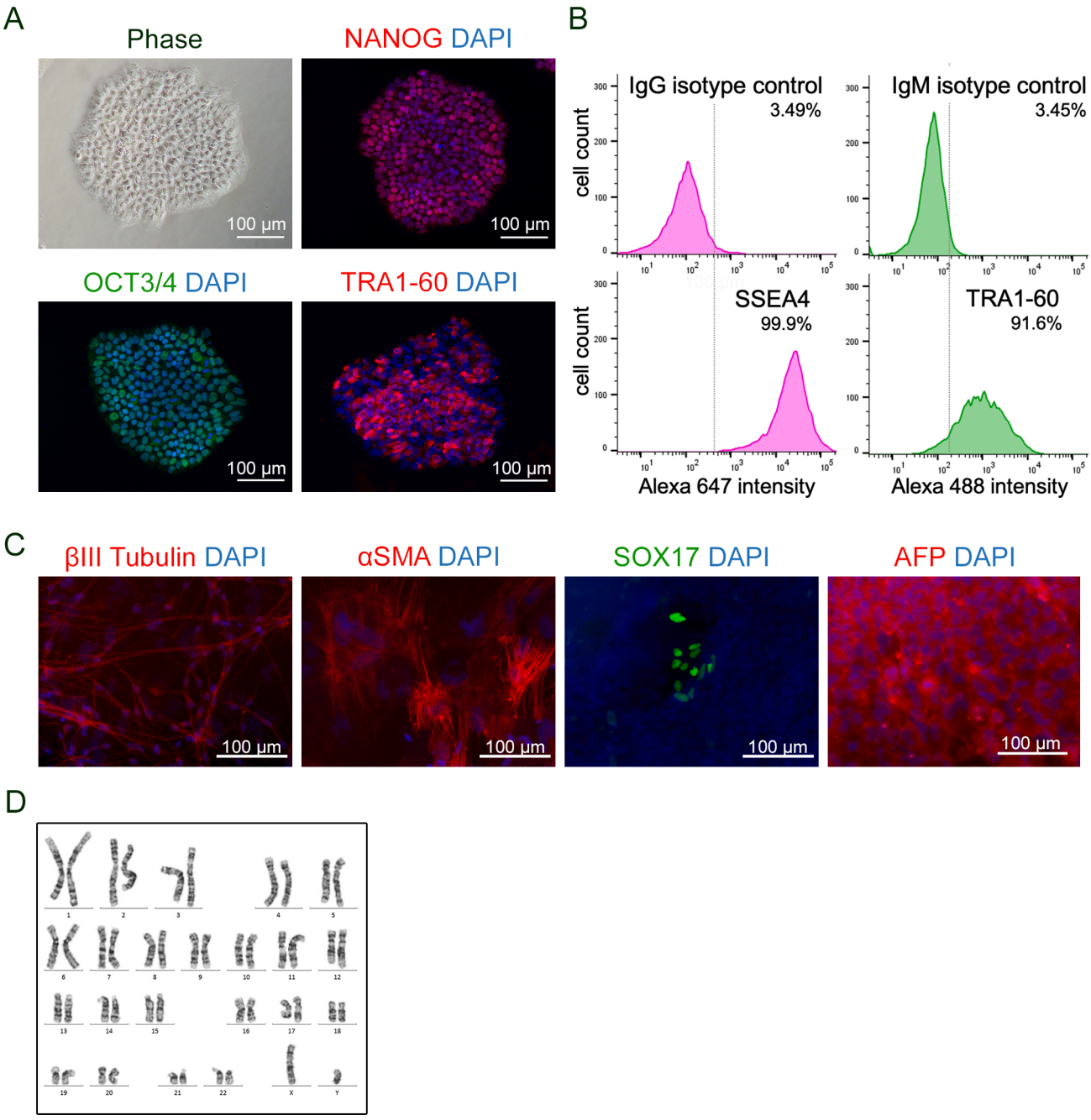

Fig. 1. Characterization of the iPSC line.

4.5. Karyotype analysis

iPSCs at passage 6 were karyotyped; 80–90% confluent cells were treated with colcemid solution for at least 2 h at 37 °C and then harvested. The cells were given a hypotonic treatment and then fixed. Twenty G-banded metaphase plates were karyotyped by LSI Medience (Tokyo, Japan).

4.6. Flow cytometric analysis

iPSCs were dissociated into single cells using Accumax (Innovative Cell Technology). The dissociated cells were suspended at 1.0×10^6 cells/ml in PBS containing 2% FBS and stained with Alexa-647 conjugated SSEA-4 monoclonal antibody or with Alexa-488 conjugated TRA1-60 monoclonal antibody (BD Biosciences, San Jose, CA) for 30 min at 4 °C. Alexa-647 conjugated isotype control or Alexa-488 conjugated isotype control was used as a negative control. After staining with the antibody, the cells were washed twice in PBS with 2% FBS and analyzed using FACS Aria (BD Biosciences).

4.7. DNA fingerprinting

Short tandem repeat (STR) analysis was performed using a PowerPlex 16 HS system (Promega, Madison, WI, US) and 3500xL Genetic Analyzer. PowerPlex 4C Matrix Standard (Promega) was used for calibration of the system.

4.8. Mycoplasma test

Confirmation that the generated iPSCs were mycoplasma negative

Table 2
Reagent details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency markers	Rabbit anti-NANOG	1:500	Cosmo Bio Co Cat# RCAB0003P, RRID: AB_1962353
Pluripotency markers	Mouse anti-TRA1-60	1:200	Cell Signaling Technology Cat# 4746S, RRID: AB_2119059
Pluripotency markers	Mouse anti-OCT3/4	1:1000	SantaCruz Cat# sc5279, RRID: AB_628051
Ectoderm differentiation markers	Mouse anti-βIII-tubulin	1:1000	Millipore Cat# MAB1637, RRID: AB_2210524
Mesoderm differentiation markers	Mouse anti-αSMA	1:500	DAKO Cat# M0851, RRID: AB_2223500
Endoderm differentiation marker	Goat anti-SOX17	1:1000	R&D Systems Cat# AF1924, RRID: AB_355060
Endoderm differentiation marker	Mouse anti-α-Fetoprotein (AFP) Clone C3	1: 400	Sigma-Aldrich Cat# A8452, RRID: AB_258392
Secondary antibody	Donkey anti-Goat IgG Alexa Fluor 488	1:1000	Molecular Probes Cat# A-11055, RRID: AB_142672
Secondary antibody	Goat anti-Mouse IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11030, RRID: AB_2534089
Secondary antibody	Goat anti-Mouse IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-32723 RRID: AB_2633275
Secondary antibody	Goat anti-Rabbit IgG Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11010 RRID: AB_2534077
Pluripotency marker	Alexa Fluor 647 Mouse anti-SSEA4	1 μg/test	BD Biosciences Cat# 560796 RRID: AB_2033991
Isotype control	Alexa Fluor 647 Mouse IgG3, k Isotype Control	1 μg/test	BD Biosciences Cat# 560803 RRID: AB_2034029
Pluripotency marker	Alexa Fluor 488 Mouse anti-TRA1-60	1 μg/test	BD Biosciences Cat# 560173, RRID: AB_1645379
Isotype control	Alexa Fluor 488 Mouse IgM, k Isotype Control	1 μg/test	BD Biosciences Cat# 562,409 RRID: AB_11153124

Primers

	Target	**Forward/Reverse primer (5′-3′)**
Nested-PCR, 1st step	mycoplasma detection	*ACACCATGGGAG(C/T)TGGTAAT/ CTTC(A/T)TCGACTT(C/T) CAGACCCAAGGCAT*
Nested-PCR, 2nd step	mycoplasma detection	*GTG(C/G)GG(A/C)TGGATCACCTCCT/ GCATCCACCA(A/T)A(A/T)AC(C/T)CTT*
Episomal plasmids integration PCR	OriP	*TTCCACGAGGGTAGTGAACC/ TCGGGGGTGTTAGAGACAAC*
Endogenous allele detection PCR	human Fbx15	*GCCAGGAGGTCTTCGCTGTA/ AATGCACGGCTAGGGTCAAA*
qPCR, human pluripotent gene expression	human OCT3/4 (CDS)	*CCCCAGGGCCCCATTTTGGTACC/ ACCTCAGTTTGAATGCATGGGAGAGC*
qPCR, transgene expression	human OCT3/4 (Tg)	*CATTCAAACTGAGGTAAGGG/ TAGCGTAAAAGGAGCAACATAG*
qPCR, human pluripotent gene expression	human SOX2 (CDS)	*TTCACATGTCCCAGCACTACCAGA/ TCACATGTGTGAGAGGGGCAGTGTGC*
qPCR, transgene expression	human SOX2 (Tg)	*TTCACATGTCCCAGCACTACCAGA/ TTTGTTTGACAGGAGCGACAAT*
qPCR, human pluripotent gene expression	human KLF4 (CDS)	*ACCCATCCTTCCTGCCCGATCAGA/ TTGGTAATGGAGCGGCGGGACTTG*
qPCR, transgene expression	human KLF4 (Tg)	*CCACCTCGCCTTACACATGAAGA/ TAGCGTAAAAGGAGCAACATAG*
qPCR, human pluripotent gene expression	human L-MYC (CDS)	*GCGAACCCAAGACCCAGGCCTGCTCC/ CAGGGGGTCTGCTCGCACCGTGATG*
qPCR, transgene expression	human L-MYC (Tg)	*GGCTGAGAAGAGGATGGCTAC/ TTTGTTTGACAGGAGCGACAAT*
qPCR, human pluripotent gene expression	human LIN28 (CDS)	*AGCCATATGGTAGCCTCATGTCCGC/ TCAATTCTGTGCCTCCGGGAGCAGGGTAGG*
qPCR, transgene expression	human LIN28 (Tg)	*AGCCATATGGTAGCCTCATGTCCGC/ TAGCGTAAAAGGAGCAACATAG*

was provided using the Vero-Hoechst and nested-PCR (polymerase chain reaction) methods. For the Vero-Hoechst method, the culture medium was stained using Hoechst33258 (Thermo Fisher Scientific) after co-culture with VERO cells (RCB0001, RIKEN BRC Cell Bank). Primers used in the nested-PCR are listed in Table 2.

4.9. Detection of transgenes

Loss of transgene expression and lack of integration were validated at passage 10 by quantitative PCR (qPCR) and PCR analyses, respectively (Supplementary Fig. 2, Okita et al., 2013). Total RNA was purified from iPSCs with QIAzol (Qiagen, Hilden, Germany) and an miRNA mini kit (Qiagen); 1 μg total RNA was reverse transcribed with SuperscriptIII (Thermo Fisher Scientific) and dT20 primer, according to the manufacturer's instructions. qPCR was performed with TB Green Premix Ex Taq™ II (Takara Bio, Kusatsu, Japan) and analyzed with the QuantStudio™ 12 K Flex Real-Time PCR System (Applied Biosystems, Foster City, CA). Genomic DNA was isolated from iPSCs using protenase K (Takara Bio). Amplification was carried out using ExTaq (Takara Bio). Primers are listed in Table 2.

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.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.scr.2021.102274.

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