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Lab Resource: Stem Cell Line

Induced pluripotent stem cells derived from a patient with familial idiopathic basal ganglia calcification (IBGC) caused by a mutation in SLC20A2 gene

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A B S T R A C T

Idiopathic basal ganglia calcification (IBGC), also known as Fahr disease or primary familial brain calcifications (PFBC), is a rare neurodegenerative disorder characterized by calcium deposits in basal ganglia and other brain regions, causing neuropsychiatric and motor symptoms. We established human induced pluripotent stem cells (iPSCs) from an IBGC patient. The established IBGC-iPSCs carried SLC20A2 c.1848G→A mutation (p.W616* of translated protein PiT2), and also showed typical iPSC morphology, pluripotency markers, normal karyotype, and the ability of in vitro differentiation into three-germ layers. The iPSC line will be useful for further elucidating the pathomechanism and/or drug development for IBGC.

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Resource Table

| Unique stem cell line identifier | CIRA003-A |
| Alternative name of stem cell line | HPS1036 |
| Institution | Center for iPS Cell Research and Application (CiRA), Kyoto University |
| Contact information of distributor | Haruhisa Inoue |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | 28 year-old, male |
| Cell source | Peripheral blood mononuclear cells (PBMCs) |
| Method of reprogramming | Episomal vectors (SOX2, KLF4, OCT4, L-MYC, LIN28 and p53 carboxy-terminal dominant-negative fragment) |
| Associated disease | Idiopathic basal ganglia calcification (IBGC) |
| Gene/locus | SLC20A2 |
| Method of modification | Not available |
| Gene correction | NO |
| Name of transgene or resistance | Not available |

Inducible constitutive system: Not available
Date archived stock date: Jan, 2015
Cell line repository/bank: RIKEN BioResource Center, Japan
Ethical approval: Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University (approved No. R0091 and G259), and Gifu university (approved No. 25-65)

Resource utility

IBGC is a rare disease, characterized by massive calcification around brain vessels and various neurological symptoms, the mechanism of which is unknown. IBGC-iPSC will provide endothelial cells or neural cells and help the elucidation of the pathomechanism in the neurovascular system, one of key targets to treat neurological disorders.

Resource details

Idiopathic basal ganglia calcification (IBGC) is a rare genetic condition characterized by symmetric calcification in the basal ganglia and other brain regions. The clinical symptoms of IBGC include neuropsychiatric...
symptoms, dementia, psychosis, seizures, or chronic headache, and normal serum levels of calcium, phosphate, alkaline phosphatase and parathyroid hormone. Typical age at clinical onset is between 20 and 50 years, and most individuals are asymptomatic (Nicolas et al., 2015). The neuropathological hallmark of IBGC is calcification of peri- and intra-vascular space of capillaries in the basal ganglia, dentate nuclei of the cerebellum and white matter. IBGC is frequently inherited in an autosomal dominant manner. Approximately half of the cases of familial IBGC were showed in the cerebellum and white matter. IBGC is frequently inherited in an autosomal dominant manner. Approximately half of the cases of familial IBGC are caused by mutations in SLC20A2 (Yamada et al., 2014). The SLC20A2 coding region was amplified from genomic DNA and retrotranscribed into complementary DNA using reverse transcriptase. The complementary DNA was subjected to PCR amplification and sequenced. The mutations predicted to result in a loss of function of SLC20A2 were confirmed by genotyping by sequencing analysis.

Table 1
Characterization and validation.

| Classification   | Test                                      | Result                                               | Data          |
|------------------|-------------------------------------------|------------------------------------------------------|---------------|
| Morphology       | Photography                               | Similar to human embryonic stem cells                | Fig. 1 panel A|
| Phenotype        | Immunochemistry                           | Assess staining of pluripotency markers: NANOG and SSEA-4 | Fig. 1 panel A|
|                  | Flow cytometry                            | SSEA-4 87.6%                                        | Fig. 1 panel E|
| Genotype         | Karyotype (G-banding) and resolution      | 46XY, Resolution 450–500                            | Fig. 1 panel C|
| Identity         | Microsatellite PCR (mpCR)                 | Not performed                                       | Not performed |
|                  | STR analysis                              | 16 loci, matched                                    | Supplementary Table 1 |
| Mutation analysis | Sequencing                               | Heterozygous, SLC20A2 mutation (c.1848G-->A, p.W616* of translated protein PIT2) | Fig. 1 panel D|
| Genotype         | Southern Blot OR WGS                      | Not performed                                       | Not performed |
| Microbiology and virology | Mycoplasma                              | Mycoplasma testing by luminescence method (MycoAlert™) | Not shown but available |
|                  |                                         | Mycoplasma detection kit                             | with author    |
| Differentiation potential | Embryoid body formation OR Teratoma formation OR Scorecard | Describe expression of genes in embryoid bodies: SOX-17, αSMA, and βIII-tubulin | Fig. 1 panel B|
| Donor screening  | HIV 1 + 2 Hepatitis B, Hepatitis C        | HIV1 (negative), HIV2 (not performed), Hepatitis B (negative), and Hepatitis C (negative) | Not shown but available with author |
| Genotype Additional info | Blood group genotyping                    | Not available                                        | Not available |
|                  | HLA tissue typing                         | Not available                                        | Not available |

Materials and methods

Ethics statements

Generation and use of human iPSCs was approved by the Ethics Committee of the Department of Medicine and Graduate School of Medicine, Kyoto University and Gifu University, and all methods were performed in accordance with the approved guidelines. Formal informed consent was obtained from the patient.

Establishment of iPSCs

Peripheral blood mononuclear cells (PBMCs) from an IBGC patient were cultured in StemFit/AK03 A, B without C (Ajinomoto, Tokyo, Japan) supplemented with IL-6, SCF, TPO, Flt-3L, IL-3, G-CSF (Wako Pure Chemical Industries, Osaka, Japan). After 7 days of culture, reprogramming factors, including SOX2, KLF4, OCT4, L-MYC, LIN28 and p53 carboxy-terminal dominant-negative fragment, were transduced into PBMCs with episomal vectors (Okita et al., 2013). PBMCs were cultivated in disseminated onto MEF feeder-cell with Primate ES cell medium (ReproCELL Inc., Yokohama, Japan). iPSCs colonies were picked up, and the iPSC maintenance method was changed to a feeder-free system by using StemFit medium (AK02N, Ajinomoto, Tokyo, Japan) on iMatrix-511 (Nippi, Tokyo, Japan)-coated plates.

Genotyping by sequencing analysis

Genomic DNA was extracted from PBMCs and iPSCs with Purelink Genomic DNA Kits (Invitrogen, Thermo Fisher Scientific, Waltham, MA). The SLC20A2 coding region was amplified by using KOD-plus-Neo (Toyobo, Osaka, Japan) and Veriti Thermal Cycle (Thermo Fisher Scientific), followed by direct sanger sequence (3700 Genetic Analyzer; formerly Applied Biosystems, Thermo Fisher Scientific).

In vitro differentiation

The established iPSCs were harvested by TrypLE express (Thermo Fisher Scientific) and cultivated in DMEM/F12/Glutamax (Thermo Fisher Scientific), 20% knockout serum replacement (KSR; Thermo Fisher Scientific), 10 μM Y-27632 (Nacalai tesque, Kyoto, Japan) on 0.5% Lipidure (Nichiyu, Tokyo, Japan)-coated U-shape-bottom 96-well-plates (Greiner bio–one, Kremsmünster, Austria) for generation of aggregates. On day 7, aggregates were seeded onto matrigel-coated 96-well-plates and differentiated into three germ layer in DMEM plus 10% fetal bovine serum (Thermo Fisher Scientific).

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde and blocked with PBST containing 5% Blocking One Histo (Nacalai tesque, Kyoto, Japan). DAPI (Thermo Fisher Scientific) was used for nuclei. Fluorescence imaging was performed by fluorescence microscope BIOREVO BZ-9000 (Keyence, Osaka, Japan).

Karyotype analysis

Karyotyping was performed by LSI Medience Corporation (Tokyo, Japan).
Table 2
Summary of antibodies and primers.

| 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-SSEA-4 | 1:1000 | Millipore Cat# MAB4304, RRID: AB_177629 |
| Ectoderm differentiation markers                        | Mouse anti-βIII-tubulin | 1:500 | Millipore Cat# CBL412X, RRID: AB_1977541 |
| Mesoderm differentiation markers                        | Mouse anti-αSMA | 1:100 | DAKO Cat# M0851, RRID: AB_2223500 |
| Endoderm differentiation markers                        | Goat anti-SOX17 | 1:300 | R and D Systems Cat# AF1924, RRID: AB_355006 |
| Secondary antibodies                                   | Donkey anti-Goat IgG Alexa Fluor 488 | 1:1000 | Thermo Fisher Scientific Cat# A-11055, RRID: AB_142672 |
| Secondary antibodies                                   | Goat anti-Rabbit IgG Alexa Fluor 488 | 1:1000 | Thermo Fisher Scientific Cat# A-11034, RRID: AB_2576217 |
| Secondary antibodies                                   | Goat anti-Mouse IgG Alexa Fluor 546 | 1:1000 | Thermo Fisher Scientific Cat# A-11030, RRID: AB_2534089 |
| Primers                                                | SLC20A2  | Forward/Reverse primer (5'-3') | GCTGAAGAGAAGAATCCCCAAAC/Rv:GGTGAACACTGGGATGGCAG |

Flow cytometry analysis

iPSCs were dissociated into single cell by using Accumax (Innovative Cell Technologies, San Diego, CA), and were incubated at 1.0 × 10^6 cells/ml in PBS with 2% FBS and 20 μl SSEA-4 APC conjugated monoclonal antibody (BD Biosciences, Franklin Lakes, NJ) for 30 min at 4 °C. After staining, the cells were washed twice in PBS with 2% FBS and were analyzed on a FACS Aria II (BD Biosciences) high-speed cell sorter using 647 nm excitation and 100 μm nozzle. Unstained controls were also analyzed as negative control to exclude data from non-specific fluorescence.

DNA fingerprinting

STR analysis was performed by using AuthentiFiler PCR Amplification Kit (Thermo Fisher Scientific).

Mycoplasma test

iPSCs were confirmed to be mycoplasma-negative using the MycoAlert kit (Lonza, Basel, Switzerland) in accordance with the manufacturer’s instructions.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2017.07.028.

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Fig. 1. Characterization of the iPSC line. (A) Established IBGC-iPSCs displayed a typical round-shaped colony. Immunocytochemical staining of iPSCs showed positive staining of pluripotency markers NANOG (green) and SSEA-4 (red). Scale bars: 200 μm. (B) Spontaneously differentiated cells after embryoid body formation showed positive staining for each marker of three-germ layer: βIII-tubulin (red), alpha smooth muscle actin: αSMA (red), and SOX17 (green). Scale bars: 100 μm. (C) Karyotype analysis of patient iPSCs showed a normal karyotype of 46 XY. (D) Sanger sequence of the SLC20A2 gene in iPSCs showed a heterozygous c.1848G→A (p.W616*) mutation. (E) FACS analysis with evaluation of SSEA-4 staining.