Generation of 10 patient-specific induced pluripotent stem cells (iPSCs) to model Pitt-Hopkins Syndrome

S.R. Sripathy, Y. Wang, R.L. Moses, A. Fatemi, D.A. Batista, B.J. Maher

Abstract

Autosomal dominant mutations in transcription factor 4 (TCF4) are associated with a rare syndromic form of Autism Spectrum Disorder (ASD) called Pitt-Hopkins Syndrome (PTHS). Here, we report the generation of a collection of induced pluripotent stem cells (iPSCs) from 5 patients diagnosed with PTHS and 5 familial controls. These patient-derived iPSCs contain a variety of mutations within the TCF4 gene, possess a normal karyotype and express all the appropriate pluripotent stem cell markers. These novel patient lines will be a useful resource for the research community to study PTHS and the function of TCF4.

Keywords

Induced pluripotent stem cells (iPSCs); Pitt-hopkins syndrome; Transcription factor 4 (TCF4); Autism Spectrum Disorder (ASD)
1. Resource Table:

| Unique stem cell lines identifier | LIBD010-A            |
|-----------------------------------|----------------------|
|                                   | LIBD011-A            |
|                                   | LIBD012-A            |
|                                   | LIBD013-A            |
|                                   | LIBD014-A            |
|                                   | LIBD015-A            |
|                                   | LIBD016-A            |
|                                   | LIBD017-A            |
|                                   | LIBD018-A            |
|                                   | LIBD019-A            |
| Alternative names of stem cell lines | PTHS1022.05 (LIBD011-A) |
|                                   | PTHS1023.05 (LIBD012-A) |
|                                   | PTHS3001.04 (LIBD016-A) |
|                                   | PTHS3004.08 (LIBD019-A) |
|                                   | PTHS1025.05 (LIBD014-A) |
|                                   | WT1021.01 (LIBD010-A) |
|                                   | WT1024.01 (LIBD013-A) |
|                                   | WT3002.01 (LIBD017-A) |
|                                   | WT3003.06 (LIBD018-A) |
|                                   | WT1026.01 (LIBD015-A) |
| Institution                       | Lieber Institute for Brain Development |
| Contact information of distributor | Dr. Brady Maher brady.maher@libd.org |
| Type of cell lines                | iPSC                 |
| Origin                            | Human                |
| Cell Source                       | Dermal Fibroblasts   |
| Clonality                         | Clonal               |
| Method of reprogramming           | Sendai Virus System  |
| Multiline rationale               | Control Disease Pairs with varying mutations |
| Gene modification                 | Yes                  |
| Type of modification              | Congenital Mutation  |
| Associated disease                | Pitt- Hopkins Syndrome (PTHS) |
| Gene/locus                        | PTHS1022 – missense – p.Arg76Ter |
|                                   | PTHS1023 – missense – C.1153C > T |
|                                   | PTHS3001 – splice c.922 + 3G > T |
|                                   | PTHS3004 – deletion Arr18q21.2q21.32 |
|                                   | PTHS1025 – deletion 18q21.2q21.31 |
| Method of modification            | N/A                  |
| Name of transgene or resistance   | N/A                  |
| Inducible/constitutive system     | N/A                  |
| Date archived/stock date          | N/A                  |
| Cell line repository/bank         | https://hpscreg.eu/search?q=LIBD |
| Ethical approval                  | JHU ISCRO Committee (Approval Number: RN00000211 For ISCRO000000385) |

2. Resource utility

PTHS-specific and familial control iPSC lines can be used to model PTHS in vitro by differentiating disease specific tissues in order to study disease mechanisms that may lead to future therapeutic interventions (Table 1).
3. Resource details

Autism Spectrum Disorder (ASD) encompasses a wide range of individuals with mild to severe symptoms, including severe impairments in communication and language, difficulties in social engagement, fascinations with specific objects, and stereotypical repetitive motor behaviours. Pitt-Hopkins Syndrome (PTHS) is a relatively understudied syndromic form of ASD caused by mutation or deletion in TCF4 resulting in a dominant negative TCF4 protein and/or haploinsufficiency which leads to abnormal brain development. Patients with TCF4 mutations have profound developmental delays, autistic behaviours, and gastrointestinal abnormalities. TCF4 is a basic helix-loop-helix (bHLH) transcription factor that has been associated with a number of disorders, including PTHS, schizophrenia, major depression, 18q deletion syndrome, Edwards Syndrome (trisomy 18) and Fuchs corneal dystrophy. TCF4 regulates many important functions during cortical development including neural progenitor proliferation, lineage commitment, neuronal migration, columnar organization, neurite outgrowth and myelination (Li et al., 2019; Page et al., 2018; Phan et al., 2020). In addition, TCF4 appears to be an activity-dependent transcription factor that is capable of responding to neuronal activity while also regulating neuronal activity (Page et al., 2018; Rannals et al., 2016; Sepp et al., 2017).

To improve cellular modelling of ASD and for studying the role of TCF4 in human cortical development, we have reprogrammed, established and validated 5 PTHS patient-derived and 5 parental control-derived induced pluripotent stem cell (iPSC). Expression of pluripotency markers (Nanog, Sox2 and Tra 1–60) at protein level was validated by immunocytochemistry (Fig. 1A-J; column 4–6) and markers (Nanog, Oct4, Sox2 and Lin28A) at transcript level by qPCR (Fig. S1A′-J′). In vitro directed differentiation followed by immunocytochemistry for endoderm (Sox17), mesoderm (Brachyury) and ectoderm (Pax6) markers showed differentiation potential into cells of all three germ layers (Fig. 1A-J; column 1–3). Additionally, karyotyping analysis (Fig. S1A-J) was performed for all 10 reprogrammed iPS cell lines. These lines are now freely available to the research community. Sub-clone pairs for the reported lines are also available (Table 2).

4. Materials and methods

4.1. Reprogramming and hiPSC maintenance

Early passage fibroblasts (fewer than 5 passages) were thawed and cultured in cDMEM media (DMEM (11960044; Thermo Fisher Scientific), 10% Fetal Bovine Serum (16140071; Thermo Fisher Scientific), 1% Non-Essential Amino Acids (11140050; Thermo Fisher Scientific), 0.1% beta-mercaptoethanol (21985023; Thermo Fisher Scientific) and 1% penicillin/streptomycin (15140122; Thermo Fisher Scientific)) for 2 days prior to infection. All reprogramming was performed using CytotuneTM-iPS 2.0 Sendai Reprogramming Kit (A16517; Thermo Fisher Scientific), according to the manufacturer's protocol. Single hand-picked clones were cultured onto an irradiated mouse embryonic fibroblast (ir-MEF) feeder layer in hESC media (DMEM/F-12 (113330032; Thermo Fisher Scientific), 20% Knockout serum replacement (A3181502; Thermo Fisher Scientific), 1% Non-essential amino acid, 0.2% b-mercaptoethanol) supplemented with 10 ng/ml FGF2 (100-18B; Peprotech). The culture medium was changed daily. iPS cells were passaged once a week using 1 mg/ml
collagenase (17104019; Thermo Fisher Scientific). All iPS cells were cultured at 37 °C, 5% CO₂ and 95% humidity.

4.2. **Karyotype analysis**

iPS cells were cultured in T25 flasks for shipping purposes at passage 9. Karyotype assay and analysis were performed for all generated iPSC lines at WiCell.

4.3. **qPCR analysis**

Quantitative analysis of pluripotency markers was performed (Table 3). Total RNA was purified from iPS (passage 9) and ESC (passage 20) using RNeasy Mini Kit (74004; Qiagen) and converted to cDNA using High-Capacity cDNA Reverse Transcription Kit (4387406; Thermo Fisher Scientific). qPCR assays were carried out in duplicate or triplicate using the QuantStudio3 Real-Time PCR system (Applied Biosystems). The relative fold changes in expression were calculated using the 2-ΔΔCt method relative to GAPDH and fibroblasts as internal controls. Fibroblast expression was set as a reference point (scale set to 1).

4.4. **In vitro directed differentiation**

Prior to directed differentiation, iPS cell lines were converted into feeder free conditions cultured on matrigel-coated plates (Matrigel – 354234; Corning). Cells were cultured for one week prior to differentiation in StemFlex (A3349401; Thermofisher Scientific) or until 90% confluency in incubator conditions mentioned above. Directed trilineage differentiation was performed according to Manufacturer’s protocol (05230; Stem Cell Technologies). iPS cells were cultured in 24-well ibidi plates and cultured in culture media for a week. Cells were fixed on day 7 and immunocytochemistry analysis was performed as described below.

4.5. **Immunofluorescence staining**

To assess pluripotency marker expression, primary antibodies SOX2, NANOG, TRA 1–60 were used. Additionally, primary antibodies Pax6, Brachyury, and Sox17 were used to validate the tri-lineage differentiation potential (Table 3). Cells grown on 24-well treated ibidi plates were fixed with 4% paraformaldehyde (PFA; 15714-S; Electron Microscopy Sciences) at room temperature for 15 min. Fixed cells were permeabilized with 0.3% Triton X-100 blocking buffer (10% donkey serum in DPBS) for a minimum of 45 min at room temperature. Donkey serum was used to avoid non-specific binding of antibodies. The cells were incubated with primary antibodies overnight at 4 °C. After three 5 min DPBS washes, cells were incubated with fluorescence-conjugated corresponding secondary antibodies at room temperature for 2 h. Cells were counterstained with DAPI. Pluripotency markers were imaged at 10× objective on PerkinElmer Operetta high content imaging system and trilineage differentiation markers were imaged at 20X objective on a Zeiss confocal laser scanning microscope (LSM 700).

4.6. **Mycoplasma detection**

Mycoplasma test was carried out with Universal Mycoplasma Detection Kit (ATCC® 30–1012 K™) according to the manufacturer’s protocol. This kit uses a PCR-based assay.
followed by gel electrophoresis to detect presence of mycoplasma as a distinct band in the 434 bp to 468 bp range.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
A-J, column 1-3. Directed trilineage differentiation for all iPS cell lines. Pax6 (column 1, green), Brachyury (column 2, green) and Sox17 (column 3, green). Columns 4-6. Immunocytochemistry for pluripotency markers. Tra 1-60 (column 4, green), Nanog (column 5, green) and Sox2 (column 6, green). Blue: DAPI for all images.
Table 1

Summary of lines.

| iPSC line names | Abbreviation in figures | Mutation | Disease              |
|-----------------|-------------------------|----------|----------------------|
| LIBDi011-A      | PTHS1022                | missense – p.Arg76Ter | Pitt-Hopkins Syndrome |
| LIBDi012-A      | PTHS1023                | missense – C.1153C > T | Pitt-Hopkins Syndrome |
| LIBDi016-A      | PTHS1024                | splice c.922 + 3G > T | Pitt-Hopkins Syndrome |
| LIBDi019-A      | PTHS1025                | deletion Arr18q21.2q21.32 | Pitt-Hopkins Syndrome |
| LIBDi014-A      | PTHS1026                | deletion 18q21.2q21.31 | Pitt-Hopkins Syndrome |
| LIBDi017-A      | WT1021                  | familial pair – PTHS1022 | Parental Control |
| LIBDi013-A      | WT1024                  | familial pair – PTHS1023 | Parental Control |
| LIBDi017-A      | WT1025                  | familial pair – PTHS1024 | Parental Control |
| LIBDi015-A      | WT1026                  | familial pair – PTHS1025 | Parental Control |
Table 2

Characterization and validation.

| Classification | Test                                         | Result                                                                 | Data                        |
|----------------|----------------------------------------------|------------------------------------------------------------------------|-----------------------------|
| Morphology     | Photography                                  | Normal human embryonic stem cell like morphology of colonies           | Data not shown; but available with author |
| Phenotype      | Immunocytochemistry (Qualitative Analysis)   | Expression of pluripotency markers: NANOG, SOX2, TRA 1–60              | Fig. 1 A-J; columns 4–6    |
|                | Real-Time PCR (Quantitative Analysis)        | Expression of pluripotent markers NANOG, OCT4, SOX2, LIN28A            | Fig. S1 A–J’               |
| Genotype       | Karyotype (G-Banding) Band Resolution: PTHS1022.05 (400–450) PTHS1023.05 (425–575) PTHS3001.04 (375–450) PTHS3004.08 (350–425) PTHS1025.06 (375–450) WT1021.01 (425–500) WT1024.01 (425–500) WT3002.01 (475–525) WT3003.06 (425–500) WT1026.01 (400–450) | Normal karyotype for all lines | Fig. S1 A-J         |
| Identity       | Short Tandem Repeat (STR) analysis           | Tested 10 loci, all matched                                            | Data submitted with article. |
| Mutation analysis | Molecular testing was done in various CLIA certified clinical diagnostic laboratories | Mutations within TCF4 gene were identified as mentioned above. | N/A                        |
| Microbiology and virology | Mycoplasma                                  | Mycoplasma testing by PCR prior to cell banking                        | Data submitted with article. |
| Differentiation potential | Directed differentiation               | Pax6 for ectoderm, Brachyury for mesoderm, and Sox17 for endoderm     | Fig. 1 A-J; columns 1–3    |
| Donor screening | N/A                                         | N/A                                                                    | N/A                        |
| Genotype additional info | N/A                                       | N/A                                                                    | N/A                        |
Reagents details.

Table 3

| Antibodies used for Immunocytochemistry |
|----------------------------------------|
| Test | Antibody | Dilution | Company Cat No. and RRID |
|------|----------|----------|--------------------------|
| Pluripotency Marker | Goat anti- Nanog | 1:200 | R&D systems; AF1997; AB_355097 |
| Pluripotency Marker | Goat anti- Sox2 | 1:200 | R&D systems; MAB2018; AB_358009 |
| Pluripotency Marker | Mouse anti- Tra 1–60 | 1:100 | Stemgent; 09–0010; AB_1512170 |
| Ectoderm Marker | Sheep anti-Pax6 | 1:250 | R&D systems; AF8150; AB_2827378 |
| Endoderm Marker | Goat anti-Sox17 | 1:500 | R&D systems; AF1924; AB_355060 |
| Mesoderm Marker | Goat anti-Brachyury | 1:500 | R&D systems; AF2085; AB_2200235 |
| Secondary Antibody | Alexa Fluor 488 anti-goat, IgY (H + L) | 1:500 | Invitrogen; A11055; AB_253402 |
| Secondary Antibody | Alexa Fluor 488 anti-mouse, IgG (H + L) | 1:500 | Invitrogen; A21202; AB_141607 |
| Secondary Antibody | Alexa Fluor 488 anti-sheep, IgG (H + L) | 1:500 | Invitrogen; A11015; AB_2534082 |

| Primers |
|---------|
| Test | Target | Forward/Reverse primer (5'-3') |
|-------|--------|--------------------------------|
| Housekeeping (qPCR) | GAPDH | CATGAGAAGTATGACAACAGCCT/AGTCCTTCCACGATACCAAAGT |
| Pluripotency marker (qPCR) | NANOG | CAAAGGCAACAACCCTTT/TCTGCTTGAGGCTGAGGTAT |
| Pluripotency marker (qPCR) | OCT4 | CGAGCAATTTCAGCTCCTTGAA/TTGGGCACTGCAAGAACAATTC |
| Pluripotency marker (qPCR) | SOX2 | CCCGGGCGGAATATGC/TTGGCGCGCCGGAGATCAT |
| Pluripotency marker (qPCR) | LIN28A | AGTTGGTCAACATCGCATGG/AGTCCGCGTACCCGGGAT |

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