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

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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)

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CRediT authorship contribution statement

S.R. Sripathy: Investigation, Validation, Writing - original draft. **Y. Wang:** Investigation, Validation. **R.L. Moses:** Investigation, Validation, Writing - review & editing. **A. Fatemi:** Resources. **D.A. Batista:** Resources. **B.J. Maher:** Conceptualization, Supervision, Writing - review & editing.

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.2020.102001.

1. Resource Table:

Unique stem cell lines identifier	LIBDi010-A LIBDi011-A LIBDi012-A LIBDi013-A LIBDi014-A LIBDi015-A LIBDi016-A LIBDi017-A LIBDi018-A LIBDi019-A
Alternative names of stem cell lines	PTHS1022.05 (LIBDi011-A) PTHS1023.05 (LIBDi012-A) PTHS3001.04 (LIBDi016-A) PTHS3004.08 (LIBDi019-A) PTHS1025.05 (LIBDi014-A) WT1021.01 (LIBDi010-A) WT1024.01 (LIBDi013-A) WT3002.01 (LIBDi017-A) WT3003.06 (LIBDi018-A) WT1026.01 (LIBDi015-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	$PTHS_{1022}$ – missense – p.Arg76Ter $PTHS_{1023}$ – missense – C.1153C > T $PTHS_{3001}$ – splice c.922 + 3G > T $PTHS_{3004}$ – deletion Arr18q21.2q21.32 $PTHS_{1025}$ – 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 ISCRO00000385)

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% CO_2 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 iPSC (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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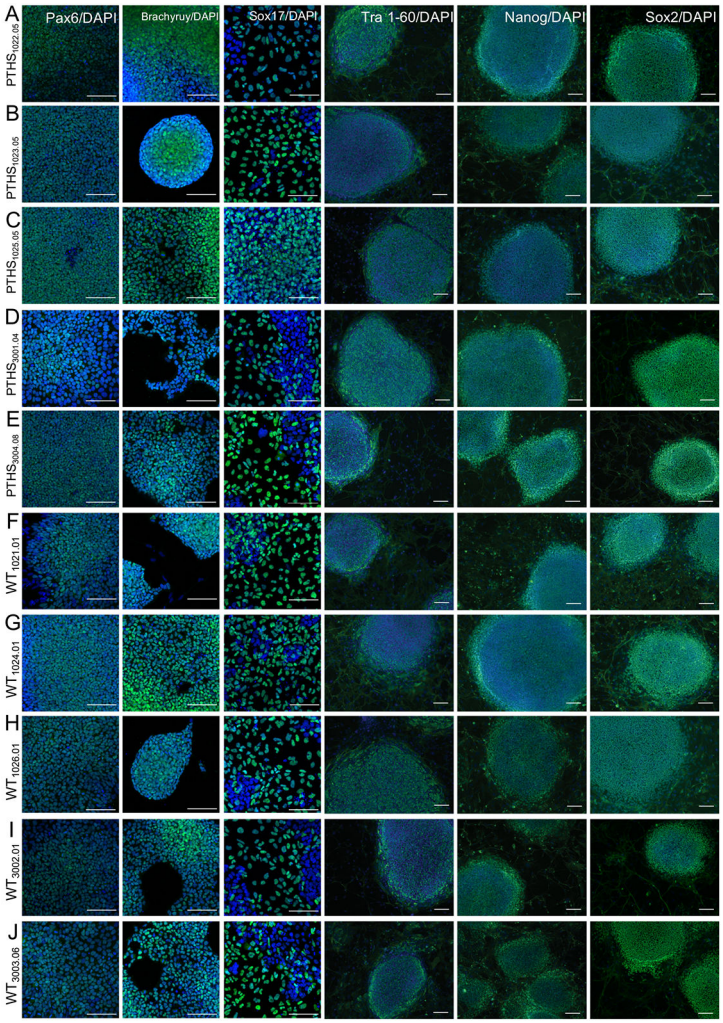

Fig. 1.

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	$PTHS_{1022}$	missense – p.Arg76Ter	Pitt-Hopkins Syndrome
LIBDi012-A	$PTHS_{1023}$	missense – C.1153C > T	Pitt-Hopkins Syndrome
LIBDi016-A	$PTHS_{3001}$	splice c.922 + 3G > T	Pitt-Hopkins Syndrome
LIBDi019-A	$PTHS_{3004}$	deletion Arr18q21.2q21.32	Pitt-Hopkins Syndrome
LIBDi014-A	$PTHS_{1025}$	deletion 18q21.2q21.31	Pitt-Hopkins Syndrome
LIBDi010-A	WT_{1021}	familial pair – $PTHS_{1022}$	Parental Control
LIBDi013-A	WT_{1024}	familial pair – $PTHS_{1023}$	Parental Control
LIBDi017-A	WT_{3002}	familial pair – $PTHS_{3001}$	Parental Control
LIBDi018-A	WT_{3003}	familial pair – $PTHS_{3004}$	Parental Control
LIBDi015-A	WT_{1026}	familial pair – $PTHS_{1025}$	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) PTHS1025.05 (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

Table 3

Reagents details.

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_2534102
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 gene (qPCR)	GAPDH	CATGAGAAGTATGACAACAGCCT/AGTCCTTCCACGATACCAAAGT	
Pluripotency marker (qPCR)	NANOG	CAAAGGCAAACAACCCACTT/TCTGCTGGAGGCTGAGGTAT	
Pluripotency marker (qPCR)	OCT4	CGAGCAATTTGCCAAGCTCCTGAA/TTCGGGCACTGCAGGAACAAATTC	
Pluripotency marker (qPCR)	SOX2	CCCCCGGCGGCAATAGCA/TCGGCGCCGGGGAGATACAT	
Pluripotency marker (qPCR)	LIN28A	AGTGGTTCAACGTGCGCATGGG/AGGTCCGGTGACACGGATGGAT	