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An induced pluripotent stem cell line (TRNDi001-D) from a Niemann-Pick disease type C1 (NPC1) patient carrying a homozygous p. I1061T (c. 3182*T>C*) mutation in the *NPC1* gene

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Abstract

Niemann-Pick disease, type C (NPC) is a rare autosomal recessive genetic disease caused by mutations in either *NPC1* or *NPC2*, which encodes an intracellular cholesterol-binding protein in lysosome. Deficiency of either *NPC1* or NPC2 protein results in malfunction of intracellular cholesterol trafficking and lysosomal accumulation of unesterified cholesterols. A human induced pluripotent stem cell (iPSC) line was generated from dermal fibroblasts of a male patient that has a homozygous p.I1061T missense mutation in *NPC1* using a non-integrating Sendai virus technique. This *NPC1* iPSC line offers a useful resource for disease modeling and drug development.

1. Resource utility

This human induced pluripotent stem cell (iPSC) line with the most frequent *NPC1* mutation, p.I1061T, is a useful tool for studies of disease phenotype and pathophysiology. It can be differentiated into various mature cell types for use as cell-based disease models of *NPC1* for compound screening and drug development.

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Declaration of Competing Interest

The authors declare no conflict of interest

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101737.

2. Resource details

Niemann-Pick disease type C1 (*NPC1*) is an inherited, progressive neurodegenerative disorder caused by mutations in *NPC1*, which encodes a lysosomal membrane protein that functions to transport cholesterol out of the endolysosomal lumen. Deficiency in *NPC1* protein results in malfunction of intracellular cholesterol trafficking and accumulation of unesterified cholesterols in late endosomes/lysosomes. The clinical manifestations of *NPC1* include hepatosplenomegaly and progressive neurodegeneration, a hallmark of the disease (Vanier, 2010). Currently, there are no effective treatments for *NPC1* disease, although a number of agents have shown the therapeutic potential for treatment of *NPC1*, including 2-hydroxypropyl-β-cyclodextrin (HPβCD) (Ottinger et al., 2014), an analog of methyl-β-cyclodextrin (MβCD) (Li et al., 2017), and miglustat (Patterson et al., 2007), etc.

In this study, a human iPSC line was established from fibroblasts of a male patient (GM18453, Coriell Institute) carrying a homozygous mutation of p. I1061T (c.3182T > *C*) in exon 21 of *NPC1* (Table 1, Fig. 1D). A non-integrating CytoTune-Sendai viral vector kit (A16517, Thermo Fisher Scientific) containing OCT3/4, KLF4, SOX2 and C-MYC pluripotency transcription factors was employed to transduce the fibroblasts using the method described previously (Chen et al., 2011). The resulting iPSC line was named TRNDi001-D that exhibited a classical embryonic stem cell morphology (Fig. 1A), normal karyotype (46, XY), as confirmed by the G-banding karyotype at passage 11 (Fig. 1C), and expressed the major pluripotent protein markers of NANOG, SOX2, OCT4, SSEA4 and TRA-1–60 (Fig. 1A, B) evidenced by both immunofluorescence staining and flow cytometry analysis. The Sendai virus vector (SeV) clearance was determined with reverse transcription polymerase chain reaction (RT-PCR) using SeV-specific primers and the vectors were eliminated by passage 15 (Fig. 1E). Mycoplasma status was confirmed to be negative (Supplementary Fig. S1) and the cell line was authenticated using a short tandem repeat (STR) DNA analysis, which demonstrated matching genotypes at all 16 loci examined (information available with the authors). Furthermore, the pluripotency of this iPSC line was confirmed by a teratoma formation experiment that exhibited its ability to differentiate into cells/tissues of all three germ layers (ectoderm: neural epithelium; mesoderm: cartilage; endoderm: gut-like tissue) *in vivo* (Fig. 1F).

3. Materials and methods

3.1. Cell culture

A patient fibroblast line (GM18453) was obtained from Coriell Cell Repositories and cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified incubator with 5% CO_2 at 37 °C. The iPSC line, TRNDi001-D, was cultured in StemFlex medium (Thermo Fisher Scientific) on Matrigel (Corning, 354277)-coated plates at 37 °C in humidified air with 5% CO_2 and 5% O_2. The cells were dissociated with Dulbecco's Phosphate Buffered Saline (DPBS) containing 0.5 mM Ethylenediaminetetraacetic acid (EDTA) and passaged when they reached 70% confluency.

3.2. Reprogramming of human skin fibroblasts

Fibroblast cells were reprogrammed into iPSCs using non-integrating Sendai virus technology following the method described previously (Chen et al., 2011).

3.3. Gene analysis of NPC1 gene

The gene analysis of variants in *NPC1* was conducted through Applied StemCell (Milpitas, California, USA). Briefly, genomic DNA was extracted from hiPSC line TRNDi001-D using QuickExtract™ DNA Extraction Solution (Lucigen) followed by PCR amplification using MyTaq™ Red Mix (Bioline, Taunton, MA). Gene amplifications were carried out using standard protocol. Genotyping of the p.I1061T mutation (c.3182 $T > C$) in exon 21 of the *NPC1* gene was performed using Sanger sequencing analysis. The specific primers for gene amplification and sequencing are listed in Table 2.

3.4. Immunocytochemistry staining

For immunofluorescence staining, patient iPSCs were fixed in 4% paraformaldehyde for 15 min, rinsed with DPBS, and permeabilized with 0.3% Triton X-100 in DPBS for 15 min. The cells were then incubated with Image-iT™ FX signal enhancer (Thermo Fisher Scientific) for 40 min at room temperature in a humidified environment and then followed by incubation individually with primary antibodies, including SOX2, OCT4, NANOG and SSEA4, diluted in the Image-iT™ FX signal enhancer blocking buffer, overnight at 4 °C. Cells were then washed and incubated with corresponding secondary antibody conjugated with Alexa Fluor 488 or Alex Fluor 594 for 1 h at room temperature (antibodies used are listed in Table 2). Cells were washed and stained with Hoechst 33342 nucleic acid stain for 15 min and imaged using an INCell Analyzer 2200 imaging system (GE Healthcare) with 20 × objective lens and Texas Red, FITC and DAPI filter sets.

3.5. Flow Cytometry analysis

The iPSCs were harvested using TrypLE Express enzyme (Thermo Fisher Scientific). Cells were fixed with 4% paraformaldehyde for 10 min at room temperature and then washed with DPBS Before fluorescence-activated cell sorting analysis, cells were permeabilized with 0.2% Tween-20 in DPBS for 10 min at room temperature and stained with fluorophore-conjugated antibodies for 1 h at 4 °C on a shaker. Relative fluorophore-conjugated animal nonimmune immunoglobulins were used as the negative control (antibodies and nonimmune immunoglobulins used are listed in Table 2). Cells were then analyzed on a BD Accuri™ C6 Flow Cytometry system (BD Biosciences).

3.6. G-banding karyotype

The G-banding karyotype analysis was conducted at WiCell Research Institute (Madison, WI, USA). Cell harvest, slide preparation, and G-banding karyotype were performed using standard cytogenetic protocols. Cells were incubated with ethidium bromide and colcemid and placed in a hypotonic solution, followed by fixation. Metaphase cell preparations were stained with Leishman's stain. A total of 20 randomly selected metaphases were analyzed by G-banding.

3.7. Short tandem repeat (STR) DNA profile analysis

Patient fibroblasts and derived iPSC line were sent to the WiCell Institute for STR DNA analysis. Briefly, the Promega PowerPlex® 16 HS System (Promega, Madison, WI) was used in multiplex polymerase chain reaction (PCR) to amplify fifteen STR loci (D5S818, D13S317, D7S820, D16S539, vWA, TH01, TPOX, CSF1PO, D18S51, D21S11, D3S1358, D8S1179, FGA, Penta D, Penta E) plus a gender determining marker, Amelogenin (AMEL). The PCR product was capillary electrophoresed on an ABI 3500xL Genetic Analyzer (Applied Biosystems) using the Internal Lane Standard 600 (ILS 600) (Promega, Madison, WI). Data were analyzed using GeneMapper® v 4.1 software (Applied Biosystems).

3.8. Testing for Sendai reprogramming vector clearance

Total RNA was isolated from TRNDi001-D iPSCs of passage 15 using RNeasy Plus Mini Kit (Qiagen). Human fibroblasts (GM05659, Coriell Institute) after infection with Sendai virus for 4 days were used as the positive control. A total of 1 ug RNA/reaction was reverse transcribed with SuperScript™ III First-Strand Synthesis SuperMix kit, and PCR was performed using Platinum II Hot-Start PCR Master Mix (Thermo Fischer Scientific). The amplifications were carried out using the following program: 94 °C, 2 min; 30 cycles of 94 °C, 15 s, 60 °C, 15 s and 68 °C, 15 s on Mastercycler pro S (Eppendorf) with the primers listed in Table 2. The products were then loaded to the E-Gel® 1.2% with SYBR Safe™ gel, and imaged by G: Box Chemi-XX6 gel doc system (Syngene, Frederick, MD)

3.9. Mycoplasma detection

Mycoplasma testing was performed and analyzed using the Lonza MycoAlert kit following the instructions from the company. Ratio B/A > 1.2 indicates mycoplasma positive. Ratio B/A 0.9–1.2 indicates ambiguous results and Ratio B/A < 0.9 indicates mycoplasma negative.

3.10. Teratoma formation assay

Human iPSCs cultured in 6- well plates were dissociated with DPBS containing 0.5 mM EDTA and approximately 1×10^7 dissociated cells were collected and resuspended in 400 μl culture medium supplemented with 25 mM HEPES (pH7.4) and stored on ice. Then, 50% volume (200 μl) of cold Matrigel (Corning, 354277) was added and mixed with the cells. The mixture was injected subcutaneously into NSG mice (JAX No. 005557) at 150 μl per injection site. Visible tumors were removed 6–8 weeks post injection and were immediately fixed in 10% Neutral Buffered Formalin. The fixed tumors were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Images were collected using the NanoZoomer Digital Pathology Software (Hamamatsu).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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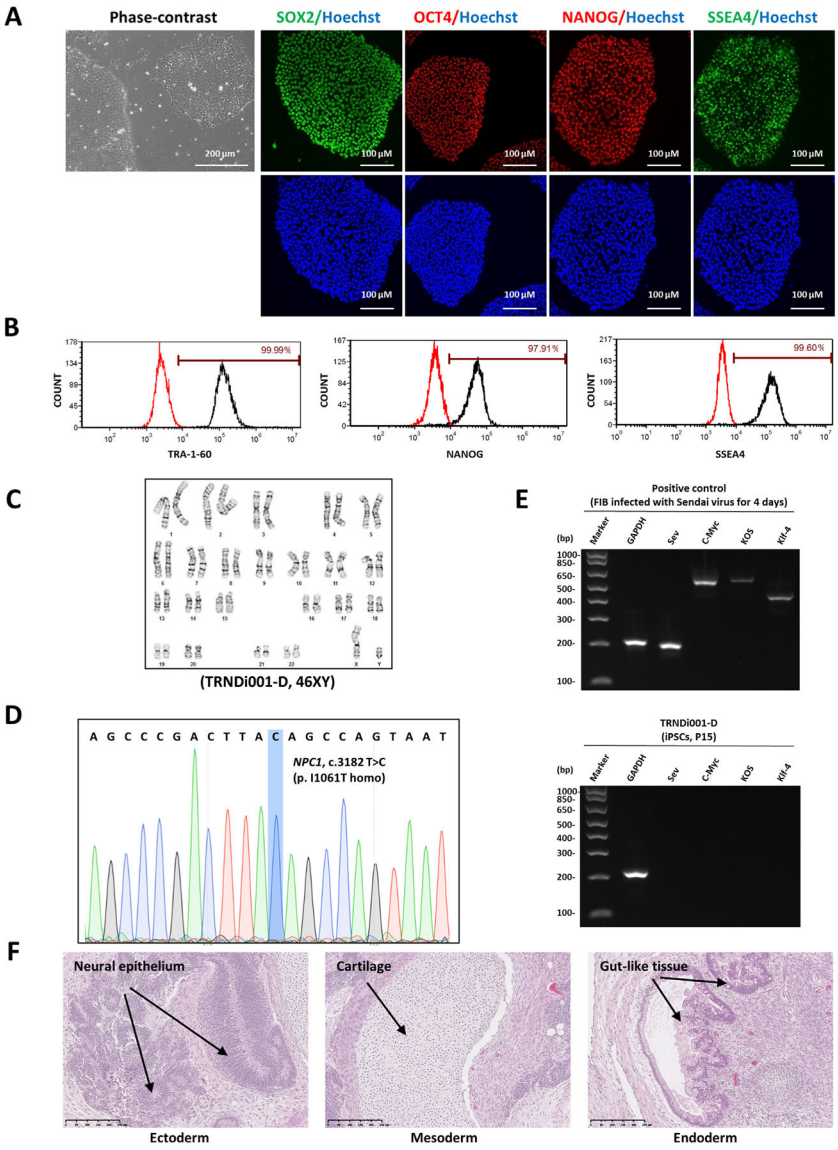

Fig. 1.

Characterization of TRNDi001-D iPSC line. **A)** Left: phase contrast imaging of TRNDi001-D colonies grown on Matrigel. Right: Representative immunofluorescent images of iPSCs positive for stem cell markers: SOX2, OCT4, NANOG, and SSEA4. Nucleus is labelled with Hoechst 33342 (blue). **B)** Flow cytometry analysis of pluripotency protein markers: TRA-1–60, NANOG, and SSEA4. **C)** Cytogenetic analysis showing a normal karyotype (46, XY). **D)** Detection of homozygous gene mutation of p. I1061T (c.3182T>C) in exon 21 of the *NPC1* gene. **E)** RT-PCR verification for the clearance of the Sendai virus from reprogrammed cells. Sendai virus vector transduced fibroblasts were used as a positive control. **F)** Pathological analysis of teratoma from TRNDi001-D iPSC, showing a normal ectodermal, mesodermal, and endodermal differentiation.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 Panel A
Phenotype	Immunocytochemistry	SOX2, OCT4, NANOG, SSEA-4	Fig. 1 Panel A
	Flow cytometry	TRA-1–60 (99.99%); NANOG (97.91%) SSEA-4 (99.60%)	Fig. 1 Panel B
Genotype	Karyotype (G-banding) and resolution	46XY Resolution: 475–525	Fig. 1 Panel C
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	16 sites tested, all sites matched	Available from the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Homozygous mutation of *NPC1*, c. 3182T> *C* (p. I1061T)	Fig. 1 Panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig. S1
Differentiation potential	Teratoma formation	Teratoma with three germlayers formation. Ectoderm (neural epithelium); Mesoderm (cartilage); Endoderm (gut-like tissue)	Fig. 1 Panel F
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-SOX2	1:50	R & D systems, Cat# MAB2018, RRID: AB_358009
Pluripotency Markers	Rabbit anti-NANOG	1:400	Cell Signaling Technology, Cat# 4903, RRID: AB_10559205
Pluripotency Markers	Rabbit anti-OCT4	1:400	Thermo Fisher Scientific, Cat# A13998, RRID: AB_2534182
Pluripotency Markers	Mouse anti-SSEA4	1:1000	Cell Signaling Technology, Cat# 4755, RRID: AB_1264259
Secondary Antibodies	Donkey anti-Mouse IgG (Alexa Fluor 488)	1:400	Thermo Fisher Scientific, Cat# A21202, RRID: AB_141607
Secondary Antibodies	Donkey anti-Rabbit IgG (Alexa Fluor 594)	1:400	Thermo Fisher Scientific, Cat# A21207, RRID: AB_141637
Flow Cytometry Antibodies	Anti-Tra-1–60-DyLight 488	1:50	Thermo Fisher Scientific, Cat# MA1–023-D488X, RRID: AB_2536700
Flow Cytometry Antibodies	Anti-Nanog-Alexa Fluor 488	1:50	Millipore, Cat# FCABS352A4, RRID: AB_10807973
Flow Cytometry Antibodies	anti-SSEA-4-Alexa Fluor 488	1:50	Thermo Fisher Scientific, Cat# 53-8843-41, RRID: AB_10597752
Flow Cytometry Antibodies	Mouse-IgM-DyLight 488	1:50	Thermo Fisher Scientific, Cat# MA1–194-D488, RRID: AB_2536969
Flow Cytometry Antibodies	Rabbit IgG-Alexa Fluor 488	1:50	Cell Signaling Technology, Cat# 4340S, RRID: AB_10694568
Flow Cytometry Antibodies	Mouse IgG3-FITC	1:50	Thermo Fisher Scientific, Cat# 11-4742-42, RRID: AB_2043894

Primers	Target	Forward/Reverse primer (5'–3')
Sev specific primers (RT-PCR)	Sev/181 bp	GGA TCA CTA GGT GAT ATC GAG C/ ACC AGA CAA GAG TTT AAG AGA TAT GTA TC
Sev specific primers (RT-PCR)	KOS/528 bp	ATG CAC CGC TAC GAC GTG AGC GC/ ACC TTG ACA ATC CTG ATG TGG
Sev specific primers (RT-PCR)	Klf4/410 bp	TTC CTG CAT GCC AGA GGA GCC C/ AAT GTA TCG AAG GTG CTC AA
Sev specific primers (RT-PCR)	C-Myc/523 bp	TAA CTG ACT AGC AGG CTT GTC G/ TCC ACA TAC AGT CCT GGA TGA TG
House-Keeping gene (RT-PCR)	GAPDH/197 bp	GGA GCG AGA TCC CTC CAA AAT/ GGC TGT CAT ACT TCT CAT GG
Targeted mutation analysis (PCR)	*NPC1/321* bp	TCC AGG GTC AGG TGA TTT TGC/ TGA AAA AGG GCA GGC TTT ACC

Resource Table.

Unique stem cell line identifier	TRNDi001-D
Alternative name(s) of stem cell line	HT307D
Institution	National Institutes of Health
	National Center for Advancing Translational Sciences
	Bethesda, Maryland, USA
Contact information of distributor	Dr. Wei Zheng Wei.Zheng@nih.gov
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: N/A
	Sex: Male
	Ethnicity: N/A
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Integration-free Sendai viral vectors
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Niemann-Pick disease, type C1 (*NPC1*)
Gene/locus	Gene: *NPC1*
	Locus: 18q11-q12
	Mutation: Homozygous, *NPC1*, c. 3182T>*C*, p. I1061T
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	2016
Cell line repository/bank	N/A
Ethical approval	NIGMS Informed Consent Form was obtained from patient at time of sample submission.
	Confidentiality Certificate: CC-GM-15–004