Generation of an induced pluripotent stem cell line (TRNDi004-I) from a Niemann-Pick disease type B patient carrying a heterozygous mutation of p.L43_A44delLA in the SMPD1 gene

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Abstract

Niemann-Pick disease type B (NPB) is a rare autosomal recessive lysosomal storage disease caused by mutations in the \textit{SMPD1} gene, which encodes for acid sphingomyelinase. A human induced pluripotent stem cell (iPSC) line was generated from dermal fibroblasts of a 1-year old male patient with NPB that has a heterozygous mutation of a p.L43_A44delLA of SMPD1 using non-integrating Sendai virus technique. This iPSC line offers a useful resource to study the disease pathophysiology and as a cell-based model for drug development to treat NPB.

Resource utility

This human iPSC line is a useful tool for studies of disease phenotype and pathophysiology, and use as a cell-based disease model for drug development to treat patients with NPB.

Resource details

Niemann-Pick disease type B (NPB) is a rare autosomal recessive, lysosomal storage disease that is caused by mutations in the \textit{SMPD1} gene, leading to a deficiency in acid sphingomyelinase in the lysosome of patient cells. This deficiency leads to an accumulation of sphingomyelin in the lysosome, resulting in hepatosplenomegaly and other symptoms in NPB patients (Levran et al., 1991; Wasserstein and Schuchman, 1993).

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Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101436.

Conflict of interest

The authors declare no conflict of interest.
In this study, a human induced pluripotent stem cell (iPSC) line TRNDi004-I was established from dermal fibroblasts of a 1-year-old male patient (Table 1). The integration-free CytoTune-Sendai viral vector kit (A16517, Thermo Fisher Scientific) containing OCT3/4, KLF4, SOX2, and c-MYC pluripotency transcription factors was used to transduce the patient fibroblasts using methods previously stated (Chen et al., 2011; Beers et al., 2015). Genetic analysis shows that this iPSC line carries a heterozygous gene mutation of p.L43_A44delLA in exon 1 of SMPD1 (Fig. 1B). The cells exhibit a classical embryonic stem cell morphology (Fig. 1A) and carry a normal karyotype (46, XY), as confirmed by G-banding karyotype analysis (Fig. 1D). Immunohistochemistry staining and flow cytometry analysis demonstrated high expression levels of major pluripotency protein markers of NANOG, SOX2, OCT4, SSEA4, and TRA-1–60 on those cells (Fig. 1A and C). Sendai virus (SeV) clearance was verified with reverse transcription polymerase chain reaction (RT-PCR) using SeV-specific primers with no virus present by passage 15 (Fig. 1E). The iPSC line was not contaminated with mycoplasma (Supplementary Fig. S1) and was authenticated using STR DNA analysis, which demonstrated matching genotype at all 18 loci examined (information available with the authors). Furthermore, the pluripotency of this iPSC line was confirmed by the teratoma formation experiment that exhibited its ability to differentiate into cells/tissues of all three germ layers (ectoderm, mesoderm and endoderm) in vivo (Fig. 1F).

Materials and methods

Cell culture

Patient skin fibroblasts were obtained from Coriell Cell Repositories (GM11097) and cultured in DMEM (Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin in a humidified incubator with 5% CO₂ at 37 °C. TRNDi004-I iPSCs were cultured in StemFlex medium (Thermo Fisher Scientific) on Geltrex (Thermo Fisher Scientific)-coated plates. The cells were maintained at 5% CO₂, 5% O₂ at 37 °C and the cells were passaged with 0.5 mM ethylenediaminetetraacetic acid (EDTA) when colonies were approximately 70% confluent.

Reprogramming of human skin fibroblasts

Fibroblast cells were reprogrammed into iPSCs using the integration-free CytoTune Sendai viral vector kit (A16517, Thermo Fisher Scientific) following the methods previously stated (Chen et al., 2011; Beers et al., 2015).

Immunocytochemistry staining

For immunofluorescence staining, patient iPSCs were fixed with 4% paraformaldehyde for 30 min at RT, permeabilized with 0.3% Triton X-100 in Dulbecco’s phosphate-buffered saline (DBPS) for 15 min, and washed with DPBS. Cells were blocked using Image-iT™ FX signal enhancer (Thermo Fisher Scientific) for 1 h and incubated with primary antibodies, including SOX2, OCT4, NANOG and SSEA4, overnight at 4 °C. Cells were then washed and incubated with corresponding secondary antibody conjugated with Alexa Fluor 488 or Alexa Fluor 594 for 1 h at room temperature (antibodies used are listed in Table 2). Cells were washed and stained with Hoechst 33342 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.

**Genome analysis of variant in SMPD1 gene**

The genome analysis of variants in the *SMPD1* gene was conducted through Applied StemCell (Milpitas, CA, USA). Genomic DNA was extracted from iPSC line TRNDi004-I followed by PCR amplification using MyTaq™ Red Mix (Bioline, Taunton, MA). Amplifications were carried out using the following program: 95 °C, 2 min; 35 cycles of [95 °C, 15 s; 60 °C, 15 s; 72 °C, elongation duration varies by amplificon size], 72 °C, 5 min; 4 °C, indefinite. PCR products were subsequently sequenced by using Sanger sequencing analysis to identify potential mutations. The specific primers for gene amplification and sequencing are listed in Table 2. The heterozygous gene mutation of p.L43_A44delLA in exon 1 of *SMPD1* was further confirmed by Codex BioSolutions, Inc. (Gaithersburg, MD).

**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 immunoglobulin was used as the negative control (antibodies and nonimmune immunoglobulin used are listed in Table 2). Cells were then analyzed on a BD AccuriC6 Flow Cytometry system (BD Biosciences).

**G-banding karyotype**

The G-banding karyotype analysis was performed at WiCell Research Institute (Madison, WI, USA). A total of 20 randomly selected metaphases were analyzed by G-banding.

**Testing for Sendai reprogramming vector clearance**

Total RNA was isolated from iPSCs TRNDi004-I of passage 15 using RNeasy Plus Mini Kit (Qiagen). Human fibroblasts (Coriell Institute, GM05659), after infection with Sendai virus for 4 days, were used as a positive control. A total of 1 μg 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) with the primers listed in Table 2. The products were then loaded into an E-Gel® 1.2% with SYBR Safe™ gel and run at 120 V electric field. Finally, the image was collected using G: Box Chemi-XX6 gel doc system from Syngene (Frederick, MD).

**Short tandem repeat (STR) DNA profile analysis**

NPB patient fibroblasts and iPSCs were sent to the John Hopkins University Genetic Resources Core Facility for STR DNA profile analysis using a Promega PowerPlex 18D Kit. The PCR product was electrophoresed on an ABI Prism® 3730xl Genetic Analyzer and data were analyzed using GeneMapper® v 4.0 software (Applied Biosystems).
**Mycoplasma detection**

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

**Teratoma formation assay**

Patient iPSCs cultured in 6-well plates were dissociated with DPBS containing 0.5 mM EDTA and approximately $1 \times 10^7$ dissociated cells were collected and re-suspended in 400 μl culture medium supplied with 25 mM HEPES (pH 7.4) and stored on ice. Then, 50% volume (200 μl) of cold Matrigel (354277, Corning) 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 that were immediately fixed in 10% Neutral Buffered Formalin. The fixed tumors were embedded in paraffin and stained with hematoxylin and eosin. Images were collected and analyzed using the NanoZoomer Digital Pathology software (Hamamatsu).

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgement**

We would like to thank Dr. Zu-xi Yu of the pathology core of the National Heart, Lung, and Blood Institute, National Institutes of Health for sectioning and staining the teratomas. We also would like to thank Research Services Section at National Center for Advancing Translational Sciences for coordinating the STR DNA analysis and mycoplasma testing services. This work was supported by the Intramural Research Program of National Center for Advancing Translational Sciences, National Institutes of Health.

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Fig. 1. Characterization of TRNDi004-I iPSC line.
A) Left: phase contrast imaging of TRNDi004-I colonies grown on Geltrex. Right: Representative immunofluorescent images of iPSCs positive for stem cell markers: SOX2, OCT4, NANOAG, and SSEA4. Nucleus is labelled with Hoechst (blue).
B) Detection of a heterozygous gene mutation of p.L43_A44delLA in the SMPD1 gene of iPSC TRNDi004-I.
C) Flow cytometry analysis of pluripotency protein markers: TRA-1–60, NANOAG, and SSEA4.
D) Cytogenetic analysis showing a normal karyotype (46, XY).
E) RT-PCR verification for the clearance of the Sendai virus from reprogrammed cells. Sendai virus vector transduced fibroblasts were used as positive control.
F) Pathological analysis of teratoma from TRNDi004-I iPSC, showing a normal ectodermal, mesodermal, and endodermal differentiation.
| **Resource table** |
|-------------------|
| **Unique stem cell line identifier** | TRNDi004-I |
| **Alternative name(s) of stem cell line** | HT222I |
| **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: 1-year-old  
Sex: Male  
Ethnicity: N/A |
| **Cell source** | Skin dermal 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 B (NPB) |
| **Gene/locus** | Gene: *SMPD1*  
Locus: 11p15.4  
Mutation: p. L43_A44delLA |
| **Method of modification** | N/A |
| **Name of transgene or resistance** | N/A |
| **Inducible/constitutive system** | N/A |
| **Date archived/stock date** | 2018 |
| **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 |
Table 1

Characterization and validation.

| Classification                  | Test                          | Result                                                                 | Data                        |
|---------------------------------|-------------------------------|------------------------------------------------------------------------|-----------------------------|
| Morphology                      | Photography                   | Normal                                                                 | Fig. 1 Panel A             |
| Phenotype                       | Immunocytochemistry           | SOX2, OCT4, NANOG, SSEA4                                               | Fig. 1 Panel A             |
|                                 | Flow cytometry                | TRA-1–60 (94.30%); NANOG (91.82%); SSEA4 (99.91%)                      | Fig. 1 Panel C             |
| Genotype                        | Karyotype (G-banding) and resolution | 46XY Resolution: 500–550                                               | Fig. 1 Panel D             |
| Identity                        | Microsatellite PCR (mPCR) OR  | Not performed                                                          | N/A                        |
|                                 | STR analysis                  | 18 sites tested, all sites matched                                     | Available from the authors  |
| Mutation analysis (If Applicable)| Sequencing                   | Heterozygous mutation of *SMPl*, p.L43_A44delLA                        | Fig. 1 Panel B             |
|                                 | 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 germ layers formation (ectoderm, mesoderm and endoderm) | Fig. 1 Panel F             |
| Donor screening (Optional)      | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A                                                                  | N/A                        |
| Genotype additional info (Optional)| Blood group genotyping   | N/A                                                                    | N/A                        |
|                                 | HLA tissue typing             | N/A                                                                    | N/A                        |
### Table 2

#### Antibodies used for immunocytochemistry/flow-cytometry

| Antibody                  | Dilution | Company        | Cat # and RRID       |
|---------------------------|----------|----------------|---------------------|
| Pluripotency markers      |          |                |                     |
| Mouse anti-SOX2           | 1:50     | R & D Systems  | MAB2018, AB_358009  |
| Rabbit anti-NANOG         | 1:400    | Cell Signaling | 4903, AB_1059205    |
| Rabbit anti-OCT4          | 1:400    | Thermo Fisher  | A13998, AB_2534182  |
| Mouse anti-SEEA4          | 1:1000   | Cell Signaling | 4755, AB_1264259    |
| Secondary antibodies      |          |                |                     |
| Donkey anti-Mouse IgG     | 1:400    | Thermo Fisher  | A21202, AB_141607   |
| Rabbit anti-Rabbit IgG    | 1:400    | Thermo Fisher  | A21207, AB_141637   |
| Flow cytometry antibodies |          |                |                     |
| Anti-Tra-1–60-DyLight 488 | 1:50     | Thermo Fisher  | MA1-023-D488X, AB_2536700 |
| Flow Cytometry Antibodies |          |                |                     |
| Anti-Nanog-Alexa Fluor 488| 1:50     | Millipore      | FCABS352A4, AB_10807973 |
| Flow cytometry antibodies |          |                |                     |
| anti-SEEA-4-Alexa Fluor 488| 1:50     | Thermo Fisher  | 53–843–41, AB_1059752 |
| Flow Cytometry Antibodies |          |                |                     |
| Mouse IgM-DyLight 488     | 1:50     | Thermo Fisher  | MA1–194-D488, AB_2536969 |
| Flow Cytometry Antibodies |          |                |                     |
| Rabbit IgG-Alexa Fluor 488| 1:50     | Cell Signaling | 4340S, AB_10694568  |
| Flow cytometry antibodies |          |                |                     |
| Mouse IgG3-FITC           | 1:50     | Thermo Fisher  | 11–4742–42, 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 TTT ACA ATC CTG ATG TGG |
| Sev specific primers (RT-PCR) Klf/4410 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 CTG GTC G/TCC ACA TAC AGT CCT GTA TGA TGA TG |
| House-Keeping gene (RT-PCR) GAPDH/197 bp | GGA CGG AGA TTC CTC CCA AAT/GGC TGT TGT CAT ACT TCT CAT GG |
| Targeted mutation analysis (PCR) SMPD1–1/559 bp | GTC AGC CGA CTA CAG CAA AGA AGG TAGG TCG AAT AAC ACC CCA CCA TCA CCA TCA G |
| Targeted mutation analysis (PCR) SMPD1–2/545 bp | CTG AAG GTG AGC ACT GAA GAG TGG TGA CAA ATC AGA AGG |
| Targeted mutation analysis (PCR) SMPD1–3/545 bp | ATC TGG AAG AGA GTG TGG GTG TGA GAA GAG GAT GC |
| Targeted mutation analysis (PCR) SMPD1–4/576 bp | TGG AAC ATC TTC TCT TCT ACT GTG AGA AGG TAA CTA GTA TCT TCA TCA G |
| Targeted mutation analysis (PCR) SMPD1–5/605 bp | GCA CAC CTG TCA ATA GCT CTC CTG AGG AGG GTG GCT GGA GAT |
| Targeted mutation analysis (PCR) SMPD1–6/6164 bp | CCT TTT TAC TCT TAT CTC CAG CCA C/GGG AAG ATG ACA TGG GAT GC |
| Antibody                          | Dilution                   | Company Cat # and RRID          |
|----------------------------------|----------------------------|--------------------------------|
| Targeted mutation analysis (PCR) | SMPD1–7/443 bp             | ACC TTA TCC ATC CCA TGT CAT CTT CC/TCT CTT CGA TCC TCA GCA GC |
| Targeted mutation analysis (PCR) | SMPD1–8/483 bp             | GTC TCC GCC TCA TCT CTC TC/ACT CAC CCT GTC CCT ATT CC       |
| Targeted mutation analysis (PCR) | SMPD1–9/490 bp             | TCC TTA ATT CTC CCT ACT AGG TGC/CCC ACC AAC TCC AGG ATA AG |
| Targeted mutation analysis (PCR) | SMPD1–10/533 bp            | CTG GCT GCT CAG TTC TTT GG/TGG TAG AGA AAC CAG AAG GTC      |
| Targeted mutation analysis (PCR) | SMPD1–11/460 bp            | CAT ACC GCA CTG GCA GCT TC/CTC CAG GAA AGA AGA AGG TC      |
| Targeted mutation analysis (PCR) | SMPD1–12/656 bp            | TGG CAG CTT CTC TAC AGG GC/TAA CAG ACT GGC AGC ATC AGG T    |