Generation of an induced pluripotent stem cell line (TRNDi030-A) from a patient with Farber disease carrying a homozygous p. Y36C (c. 107 A>G) mutation in ASAH1

Brianna M. Brooks#a, Charles D. Yeh#a, Jeanette Beersb, Chengyu Liuc, Yu-Shan Chenga, Kirill Gorshkova, Jizhong Zoub, Wei Zhenga, Catherine Z. Chenab,*

aNational Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, USA
bIPSC Core, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA
cTransgenic Core, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

# These authors contributed equally to this work.

Abstract

Farber disease is an ultra-rare lysosomal storage disease. Mutations in the N-acylsphingosine amidohydrolase (ASAH1) gene, which encodes for the enzyme acid ceramidase (ACDase), cause ceramides to accumulate in the body. A human induced pluripotent stem cell (iPSC) line TRNDi030-A was generated from fibroblasts of a male patient with a homozygous p. Y36C (c.107 A>G) variant in the second exon of the ASAH1 producing the alpha subunit of ACDase. This Farber disease iPSC line is a useful resource to study disease pathophysiology and to develop therapeutics for treatment of patients with Farber disease.

1. Resource utility

TRNDi030-A is a human induced pluripotent stem cell (iPSC) line with an ASAH1 mutation, is a patient-specific disease model for the study of Farber disease phenotypes and pathophysiology and can be used as a cell-based model for drug discovery and therapeutic development to treat patients with Farber disease.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
*Corresponding author. catherine.chen@nih.gov (C.Z. Chen).

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.2021.102387.
2. Resource details

Farber disease (FD) is a rare autosomal-recessively inherited disorder caused by mutations in the N-acylsphingosine amidohydrolase (ASAH1) gene. This gene encodes the lysosomal enzyme acid ceramidase (ACDase) responsible for breaking down ceramides into sphingosine and fatty acid. The abnormal functioning of ACDase causes lipid accumulation throughout the body including in the joints and central nervous system. This clinically manifests as painful and disabling subcutaneous nodules, joint contractures, and difficulty with speaking and breathing. (Ehlert et al., 2007) FD is associated with a spectrum of clinical symptoms; the most severe cases cause infant death and milder cases affect patients’ quality of life and life expectancy. Current treatments only focus on managing symptoms as there is no effective therapeutic solution for FD. (Sands, 2013) Investigational therapies for FD include gene therapy and enzyme replacement therapy which showed efficacy in mouse and cell models (He et al., 2017; Ramsubir et al., 2008).

This study has established a human iPSC line (TRNDi030-A) from the fibroblasts of a male patient with Farber disease (GM20015, Coriell Institute) carrying a homozygous mutation, p. Y36C (c.107 A>G), in the second exon of ASAH1 (Table 1, Fig. 1D). The FD iPSC cell line, was generated via reprogramming with the non-integrating CytoTune-Sendai viral vector kit (A16517, Thermo Fisher Scientific) containing OCT3/4, KLF4, SOX2 and C-MYC pluripotency transcription factors (Beers et al., 2015). Individual colonies were picked, expanded, and further analyzed at the cellular and genetic level to confirm successful reprogramming (Table 1). The resulting iPSC line, TRNDi030-A, exhibited classical embryonic stem cell morphology under phase contrast microscopy and expressed pluripotency markers OCT4, NANOG and SOX2 in the nuclei and SSEA4 and TRA-1-60 on the plasma membrane (Fig 1A). Quantitative analysis by flow cytometry showed a 93% (Tra-1-60), 100% (SSEA-4), and 97% (Nanog) expression rate, confirming these pluripotency markers (Fig. 1B). G-banded karyotype analysis confirmed a normal karyotype at passage 7 (46, XY) with no detectable abnormalities (Fig 1C). The genetic mutation, p. Y36C (c. 107 A>G), was validated to be consistent with the description from Coriell Institute by Sanger sequencing of the PCR product harboring the single nucleotide variant (Fig. 1D). Clearance of the Sendai virus vector (SeV) from reprogramming was determined with reverse transcription polymerase chain reaction (RT-PCR) using SeV-specific primers and the vectors were eliminated by passage 23 (Fig. 1E). Furthermore, the pluripotency of this iPSC line was confirmed by a teratoma formation experiment that verified its ability to differentiate into cells/tissues of the three germ layers (ectoderm: neural epithelium; mesoderm: cartilage; endoderm: gut-like tissue) in vivo (Fig. 1F). This iPSC line was negative for mycoplasma contamination (Supplementary Fig. S1). Finally, The STR DNA profile of TRNDi030-A matched its parental GM20015 fibroblasts at 16 loci.

3. Materials and methods

3.1. Cell culture and reprogramming

Patient fibroblasts (GM20015) were 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% CO2 at 37 °C. Patient fibroblasts
were reprogrammed using non-integrating Sendai virus technology (Beers et al., 2015). Patient iPSCs were cultured in Essential 8™ (Thermo Fisher Scientific, A1517001) medium on 0.1 mg/mL Matrigel (Corning, 354277)-coated plates at 37 °C in humidified air with 5% CO2 and 5% O2. The cells were passaged with EZ-LiFT™ (Sigma-Aldrich) at generally 1:6 ratio when they reached 70% confluency with 10 μM ROCK inhibitor.

3.2. Genome analysis
The gene analysis was conducted through Applied StemCell (Milpitas, California, USA). Genomic DNA was extracted from the hiPSC line using QuickExtract™ DNA Extraction Solution (Lucigen) followed by PCR amplification using MyTaq™ Red Mix (Bioline, Taunton, MA) on the T100 Thermal Cycler from Bio-Rad (#1861096) using the following program: 95 °C, 2 min; 35 cycles of [95 °C, 15 s; 60 °C, 15 s; 72 °C, 90 s], 72 °C 5 min; 4 °C, indefinite. Genotyping for the variant was performed using Sanger sequencing analysis. The specific primers for gene amplification and sequencing are listed in Table 2.

3.3. Immunocytochemistry
Patient iPSCs cultivated at passage 9 on a 96-well plate were fixed with 4% paraformaldehyde for 15 min. at room temperature. After washing twice with DPBS, cells were permeabilized with 0.3% Triton X-100 in DPBS for 15 min. followed by blocking buffer (Cell Staining Buffer, BioLegend) for 1 hr. The cells were then incubated with primary antibodies, diluted in the blocking buffer, overnight at 4 °C. Cells were washed twice with DPBS and incubated with secondary antibodies for 1 hr. at room temperature (Antibodies used are listed in Table 2). Cell nuclei were stained with Hoechst 33342 for 15 min. and imaged with the INCell Analyzer 2500 HS imaging system (GE Healthcare). Fiji v1.52p (Bethesda, MD, NIH) was used to produce the image montage.

3.4. Flow cytometry analysis
The iPSCs at passage 20 were dissociated, washed once with DPBS, and fixed with 4% PFA for 10 min. Cell permeabilization was then conducted with 0.2% Tween-20 in DPBS for another 10 min. at room temperature, followed by staining with fluorophore-conjugated antibodies (Table 2) for 1 hr. at 4 °C. The cells were analyzed with a BD Accuri™ C6 Flowcytometry system (BD Biosciences).

3.5. G-banding karyotype
The G-banded karyotyping analysis was performed at passage 7 by WiCell Research Institute (Madison, WI). Twenty randomly selected metaphase cells were selected for the standard cytogenetic analysis.

3.6. Short tandem repeat (STR) DNA profile analysis
STR analyses of patient fibroblasts and derived iPSCs at passage 6 were performed by WiCell Research Institute using a PowerPlex® 16 HS System.
3.7. Mycoplasma detection

The Lonza MycoAlert kit was used according to the instructions on cells at passage 23. B/A ratio > 1.2 indicates the positive sample; 0.9–1.2 indicates the ambiguous result; <0.9 indicates the negative sample.

3.8. Sendai virus detection

Total RNA of derived iPSCs at passage 23 was extracted using RNeasy Plus Mini Kit (Qiagen) and 1 μg of RNA was reverse transcribed into cDNA with Superscript™ III First-Strand Synthesis SuperMix kit. The PCR was performed using Platinum II Hot-Start PCR Master Mix (ThermoFisher Scientific) with the following amplification 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). The primers were listed in Table 2. The amplified products were loaded onto the E-Gel® 1.2% with SYBR Safe™ gel and imaged by G: Box Chemi-XX6 gel doc system (Syngene, Frederick, MD). Human fibroblasts (GM05759, Coriell Institute) transfected with Sendai virus for 4 days was used as a positive control.

3.9. Teratoma formation assay

Patient iPSCs at passage 19 were dissociated with EZ-LiFT™ and resuspended approximately 1 × 10^7 cells in 400 μL culture medium supplemented with 10 mM HEPES (pH 7.4). Afterwards, 200 μL cold Matrigel (Corning, 354277) was mixed with the cells. The cell suspension was injected subcutaneously into NSG mice (JAX No. 005557) at 150 μL per injection site. Visible tumors were harvested 6–8 weeks postinjection and immediately fixed in 10% Neutral Buffered Formalin. The fixed tumors were then embedded in paraffin, sliced, and stained with hematoxylin and eosin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We want to thank Dr. Zu-xi Yu of the pathology Core of National Heart, Lung and Blood Institute, National Institutes of Health for sectioning and staining the teratoma. We also thank the Research Services Section at National Center for Advancing Translational Sciences for coordinating the mycoplasma testing services. This work was supported by the Intramural Research Programs of the National Center for Advancing Translational Sciences, National Institutes of Health.

References

Beers J, Linask KL, Chen JA, Siniscalchi LI, Lin Y, Zheng W, Rao M, Chen G, 2015. A cost-effective and efficient reprogramming platform for large-scale production of integration-free human induced pluripotent stem cells in chemically defined culture. Sci. Rep 5 (1) 10.1038/srep11319.

Ehlert K, Frosch M, Feese N, Zander A, Roth J, Vormoor J, 2007. Farber disease: clinical presentation, pathogenesis and a new approach to treatment. Pediatr. Rheumatol, 5, 15. 10.1186/1546-0096-5-15.

He X, Dworski S, Zhu C, DeAngelis V, Solyom A, Medin JA, Simonaro CM, Schuchman EH, 2017. Enzyme replacement therapy for Farber disease: proof-of-concept studies in cells and mice. BBA Clin. 7, 85–96. 10.1016/j.bbaclin.2017.02.001. [PubMed: 28275553]

Ramsubir S, Nonaka T, Gribes CB, Carpentier S, Levade T, Medin JA, 2008. In vivo delivery of human acid ceramidase via cord blood transplantation and direct injection of lentivirus as novel treatment...
approaches for Farber disease. Mol. Genetics Metab 95 (3), 133–141. 10.1016/j.ymgme.2008.08.003.

Sands MS, 2013. Farber disease: understanding a fatal childhood disorder and dissecting ceramide biology. EMBO Mol. Med 5 (6), 799–801. 10.1002/emmm.201302781. [PubMed: 23666771]
Fig. 1.
Figure 1
### Table 1

Characterization and validation.

| Classification       | Test                                      | Result                                                                 | Data                                      |
|----------------------|-------------------------------------------|------------------------------------------------------------------------|-------------------------------------------|
| Morphology           | Photography Bright field                  | Normal                                                                 | Fig. 1 Panel A                            |
| Phenotype            | Immunocytochemistry                        | SOX2, OCT4, NANOG, SSEA-4                                             | Fig. 1 Panel A                            |
|                      | Flow cytometry                            | TRA-1-60 (93%); SSEA-4 (100%); Nanog (97%)                           | Fig. 1 Panel B                            |
| Genotype             | Karyotype (G-banding) and resolution       | 46XY Resolution: 475–525                                              | Fig. 1 Panel C                            |
| Identity             | Microsatellite PCR (mPCR) OR STR analysis | Not performed; 16 sites tested; all sites matched                      | Submitted in archive with journal         |
| Mutation analysis    | Sequencing                                | Homozygous mutation of *ASAH1*, c. 107 A>G (p. Y36C)                    | Fig. 1 Panel D                            |
|                      | Southern Blot OR WGS                       | N/A                                                                    | N/A                                       |
| Microbiology and     | Mycoplasma                                | Mycoplasma testing by luminescence. Negative                          | Supplementary Fig. S1                     |
| virology             |                                           |                                                                        |                                            |
| Differentiation      | Teratoma Formation                        | Teratoma with three germ layers formation. Ectoderm (neural epithelium); Mesoderm (cartilage); Ectoderm (gut-like tissue) | Fig. 1 Panel E                            |
|                      | HIV 1 + 2 Hepatitis B, Hepatitis C        | N/A                                                                    | N/A                                       |
| Donor screening      | Blood group genotyping                    | N/A                                                                    | N/A                                       |
| (OPTIONAL)           | HLA tissue typing                         | N/A                                                                    | N/A                                       |
| Genotype additional  |                                           |                                                                        |                                            |
| info (OPTIONAL)      |                                           |                                                                        |                                            |
### Table 2

**Reagents details.**

| Antibodies used for immunocytochemistry/flow-cytometry | Dilution | Company Cat # | RRID |
|-------------------------------------------------------|----------|---------------|------|
| **Pluripotency Markers** | | | |
| Mouse anti-SOX2 | 1:50 | R & D systems, Cat # MAB2018 | RRID: AB_358009 |
| Rabbit anti-NANOG | 1:400 | Cell Signaling Technology, Cat # 4903 | RRID: AB_10559205 |
| Rabbit anti-OCT4 | 1:400 | Thermo Fisher Scientific, Cat # A13998 | RRID: AB_2534182 |
| 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 # A21207 | RRID: AB_141607 |
| **Secondary Antibodies** | | | |
| Donkey anti-Rabbit IgG (Alexa Fluor 594) | 1:50 | Thermo Fisher Scientific, Cat # 21207 | RRID: AB_141637 |
| **Flow Cytometry Antibodies** | | | |
| Anti-Tra-1-60-DyLight488 | 1:50 | Thermo Fisher Scientific, Cat # MA1-023-D488X | RRID: AB_2536700 |
| Anti-Nanog-Alexa Fluor 488 | 1:50 | Millapore, Cat # FCABS3524 | RRID: AB_10807973 |
| Anti-SSEA-4-Alexa Fluor 488 | 1:50 | Thermo Fisher Scientific, Cat # 53-8843-41 | RRID: AB_10597752 |
| Mouse IgG3-FITC | 1:50 | Thermo Fisher Scientific, Cat# 11-4742-42 | RRID: AB_2043894 |
| Rabbit IgG-Alexa Fluor 488 | 1:50 | Cell Signaling Technologies, Cat # 4340S | RRID: AB_10694568 |
| Mouse-IgM-DyLight 488 | 1:50 | Thermo Fisher Scientific, Cat # MA1-194-D488 | RRID: AB_2536969 |
| **Primers** | | | |
| **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 |
| KOS | 528 bp | ATG CAC CGC TAC GAC GTG AGC GC/ACC TTT GAC ACA ATC CTG ATG TGG |
| KiF4 | 410 bp | TTC CTG CAT GCC AGA GCC C/AAT GTA TGG AGA GGT 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 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)** | | | |
| ASAH1 | 1285 bp | GAG ATG AGG CTG GGA TGG TA/TCC TGT TTT GTC CTC GAC AGC |
Resource Table

| Unique stem cell line identifier | TRND6030-A |
| Alternative name(s) of stem cell line | HT143A; NCATS-CL8549 |
| Institution | National Institutes of Health National Center for Advancing Translational Sciences Bethesda, Maryland, USA |
| Contact information of distributor | Dr. Catherine Chen, catherine.chen@nih.gov |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info required for human ESC or iPSC | Age: N/A |
| | Sex: Male |
| | Ethnicity if known: Tunisian |
| Cell Source | fibroblasts |
| Clonality | Clonal |
| Associated disease | Farber lipogranulomatosis; Farber disease |
| Gene/locus | Gene: ASAH1 |
| | Locus: 8p22 |
| | Mutation: Homozygous, ASAH1, c. 107A>G, p. Y36C |
| Date archived/stock date | 2021 |
| Cell line repository/bank | N/A |
| Ethical approval | The fibroblasts were purchased from Coriell Institute for Medical Research and the study is funded by NIH. Documentation of NIH funding or support, the NIH CoC Policy (NOT-OD-17-109), the NIH Grants Policy Statement (See 4.1.4.1), and subsection 301(d) of the Public Health Service Act, serve as documentation of the issuance of a Certificate for a specific study. |