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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*

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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.

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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.

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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 iPS 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

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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.

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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.

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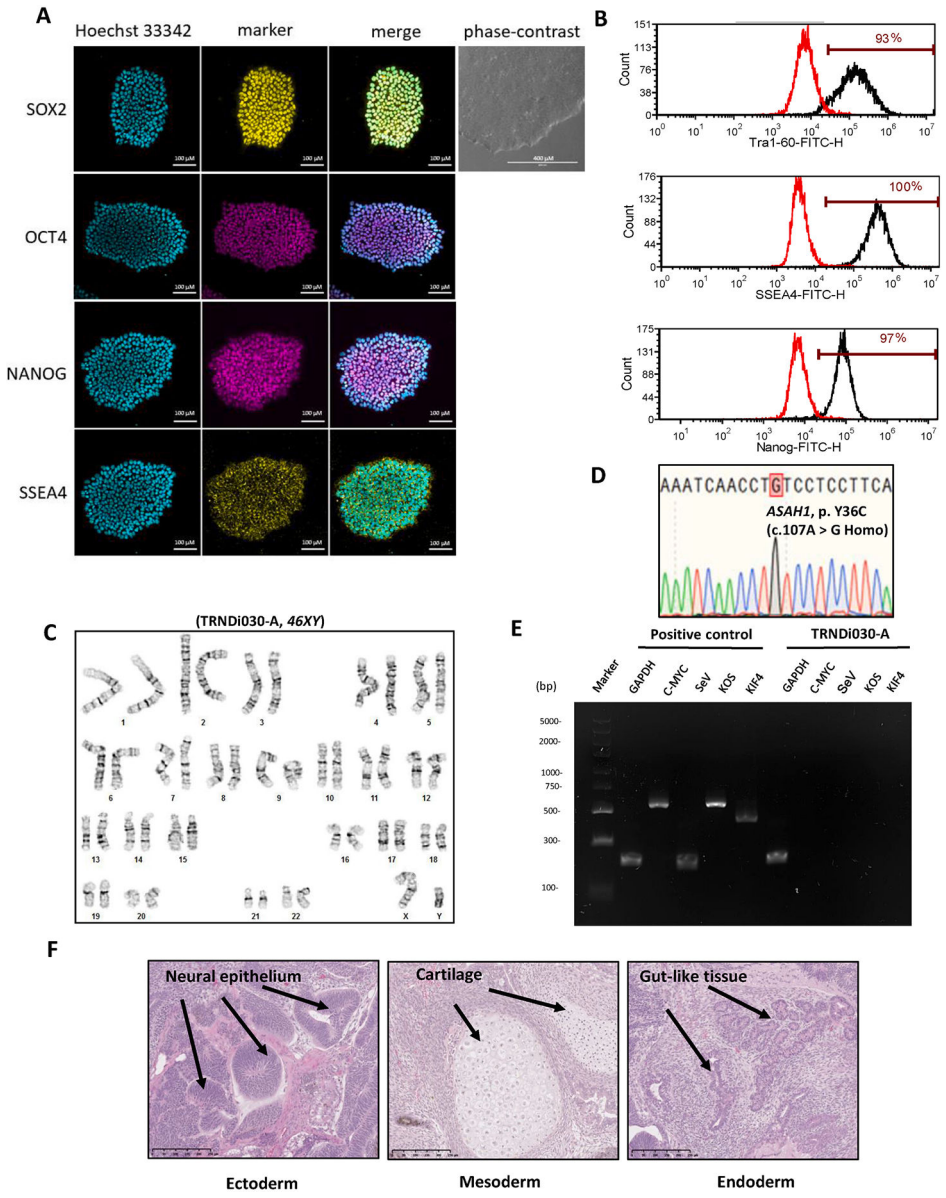

Fig. 1.
Figure 1

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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	N/A
		16 sites tested; all sites matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	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 virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig. S1
Differentiation potential	Teratoma Formation	Teratoma with three germ layers formation. Ectoderm (neural epithelium); Mesoderm (cartilage); Ectoderm (gut-like tissue)	Fig. 1 Panel E
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

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Table 2

Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**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)	1:50	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
Flow Cytometry Antibodies	Anti-Nanog-Alexa Fluor 488	1:50	Millapore, Cat # FCABS3524	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 IgG3-FITC	1:50	Thermo Fisher Scientific, Cat# 11-4742-42	RRID: AB_2043894
Flow Cytometry Antibodies	Rabbit IgG-Alexa Fluor 488	1:50	Cell Signaling Technologies, Cat # 4340S	RRID: AB_10694568
Flow Cytometry Antibodies	Mouse-IgM-DyLight 488	1:50	Thermo Fisher Scientific, Cat # MA1-194-D488	RRID: AB_2536969
	Primers			
	Target	**Size of band**	**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)	*ASAH1*	1285 bp	GAG ATG AGG CTG GGA TGG TA/TCC TGT TTT GTC CTC GAC AGC	

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Resource Table

Unique stem cell line identifier	TRNDi030-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.