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Generation of an induced pluripotent stem cell line (TRNDi002-B) from a patient carrying compound heterozygous p.Q208X and p.G310G mutations in the *NGLY1* gene

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Abstract

NGLY1 deficiency is a rare genetic disease caused by mutations in the NGLY1 gene that encodes N-glycanase 1. The disease phenotype in patient cells is unclear. A human induced pluripotent stem cell (iPSC) line was generated from skin dermal fibroblasts of a patient with NGLY1 deficiency that has compound heterozygous mutations of a p.Q208X variant (c.622C > T) in exon 4 and a p.G310G variant (c.930C > T) in exon 6 of the *NGLY1* gene. This iPSC line offers a useful resource to study the disease pathophysiology and a cell-based model for drug development to treat NGLY1 deficiency.

Resource table

Unique stem cell line identifier	TRNDi002-B
Alternative name(s) of stem cell line	HT519B
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

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Additional origin info	Age: 10-year-old Sex: Male Ethnicity: Caucasian
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Integration-free Sendai viral vectors
Genetic modification	NO
Type of modification	N/A
Associated disease	NGLY1 Deficiency
Gene/locus	$NGLY1^{Q208X}$; $NGLY1^{G310G}$
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	04-27-2018
Cell line repository/bank	Human Pluripotent Stem Cell Registry https://hpscreg.eu/cell-line/TRNDi002-B
Ethical approval	NIGMS Informed Consent Form was obtained from patient at time of sample submission. Confidentiality Certificate: CC-GM-15-004

Resource utility

This hiPSC line is a useful tool for studies of disease phenotype, disease pathophysiology, and use as a cell-based disease model for drug development to treat patients with NGLY 1 deficiency.

Resource details

NGLY1 deficiency or NGLY1-congenital disorder of deglycosylation is a rare autosomal recessive disorder caused by mutations in the *NGLY1* gene, which encodes N-glycanase 1. It cleaves N-linked glycans from glycoproteins. Deficiency in N-glycanase 1 results in malfunctions in deglycosylation of N-glycosylated proteins. Because protein glycosylation and deglycosylation play an important role in post-translational modification of proteins, deficiency of this enzyme can cause protein misfolding and aggregation in the endoplasmic reticulum and cytosol, as well as proteasome dysfunction due to defective processing of Nrf1. The typical features of NGLY1 deficiency include developmental delay or intellectual disability of varying degrees, lack of tear production, elevated liver transaminases, and a complex movement disorder (Lam et al., 1993; Suzuki, 2016; Tomlin et al., 2017).

In this study, a human induced pluripotent stem cell line was established from skin fibroblasts of a 10-year-old male patient (GM25344, Coriell Institute) carrying compound heterozygous mutations of a p. Q208X variant (c.622C > T) in exon 4 and a p.G310G variant (c.930C > T) in exon 6 of the *NGLY1* gene (Table 1). This subject is also found to be heterozygous for a c.4060 A > T mutation (Thr1354Ser) in the CACNA1S gene based on the information released by Coriell Institute, which might represent one of the risk factors for increased susceptibility to malignant hyperthermia (MH) (Beam et al., 2017). To generate the iPS cells, the 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 patient fibroblasts using the method described previously (Beers et al., 2015). The iPSC line named TRNDi002-B was generated from the patient fibroblasts. The mutations (c.622C > T and C.930C > T) of the *NGLY1* gene described by Coriell Institute were confirmed in the TRNDi002-B iPSC line by Sanger sequencing of the PCR product harboring the single nucleotide variation (SNV) (Fig. 1D). The patient iPS cells exhibited a classical embryonic stem cell morphology (Fig. 1A) and normal karyotype (46, XY), as confirmed by the G-banded karyotyping at passage 11 (Fig. 1C), 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. Sendai virus vector (SeV) clearance was detected with reverse transcription polymerase chain reaction (RT-PCR) using SeV-specific primers, the vector disappeared by passage 15 (Fig. 1E). This iPSC line was not contaminated with mycoplasma (Supplementary Fig. S1) and were authenticated using STR DNA profiling analysis, which demonstrated matching genotypes at all 18 loci examined (information available with the authors). Furthermore, the pluripotency of this iPS cell line was confirmed by the teratoma formation experiment that exhibited its ability to differentiate into cells of all three germ layers (Ectoderm, neural tube; Mesoderm, cartilage; Endoderm, gut) *in vivo* (Fig. 1F).

Materials and methods

Cell culture

Patient skin fibroblasts were purchased from Coriell Cell Repositories (GM25344) and cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified incubator with 5% CO_2 at 37 °C. Human iPS cells were cultured in StemFlex medium (Thermo Fisher) on Matrigel (Corning, 354277)-coated plates at 37 °C in humidified air with 5% CO_2 and 5% O_2. The cells were passaged with 0.5 mM Ethylenediaminetetraacetic acid (EDTA) at generally 1:6 ratio when they reach 80% confluency.

Reprogramming of human skin fibroblasts

Patient fibroblasts were reprogrammed into iPS cells using the non-integrating Sendai virus technology following the method described previously (Beers et al., 2015).

Genome analysis

The genome analysis of variants in NGLY1 was conducted through Applied StemCell (Milpitas, California, USA). Briefly, genomic DNA was extracted from hiPSC line TRNDi002-B using QuickExtract™ DNA Extraction Solution (Lucigen) followed by PCR amplification using MyTaq™ Red Mix (Bioline, Taunton, MA). Amplifications were carried out on T00 Thermal Cycler from Bio-Rad (#1861096) using the following program: 95 °C, 2 mins; 35 cycles of [95 °C, 15 s; 60 °C, 15 s; 72 °C, various length depending on size of amplicon], 72 °C 5 mins; 4 °C, indefinite. Genotyping of the compound heterozygous for a p. Q208X variant (c.622C > T) in exon 4 and a p.G310G variant (c.930C > T) in exon 6 of the *NGLY1* gene were performed using Sanger sequencing analysis. The specific primers for gene amplification and sequencing are listed in Table 2.

Immunocytochemistry

For immunofluorescence staining, patient iPSCs were fixed in 4% paraformaldehyde for 15 mins, rinsed with DPBS, and permeabilized with 0.3% Triton X-100 in DPBS for 15 mins. The cells were incubated with the Image-iT™ FX signal enhancer (ThermoFisher Scientific) for 40 mins at room temperature in a humidified environment and then followed by incubation individually with primary antibodies including SOX2, OCT4, NANOG, SSEA4 and TRA-1-60, diluted in the Image-iT™ FX signal enhancer blocking buffer, for overnight at 4 °C. After washing with DPBS, a corresponding secondary antibody conjugated with Alexa Fluor 488 or Alex Fluor 594 was added to the cells and incubated for 1 h at room temperature (Antibodies used are listed in Table 2). Cells were then stained with Hoechst 33342 for 15 mins after a wash and imaged using an INCell Analyzer 2200 imaging system (GE Healthcare) with 20× objective lens and Texas Red, FITC and DAPI filter sets.

Flow cytometry analysis

The iPSCs were harvested using TrypLE Express Enzyme (ThermoFisher Scientific). Cells were fixed with 4% paraformaldehyde for 10 mins 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 mins at room temperature and stained with fluorophore conjugated antibodies for 1 h at 4 °C on a shaker (Antibodies used are listed in Table 2). Cells were then analyzed on a BD Accuri™ C6 FlowCytometry system (BD Biosciences).

G-banding karyotype

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

Short tandem repeat (STR) analysis

Patient fibroblasts and derived iPSC lines were sent to the Johns 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® 3730x1 Genetic Analyzer and data was analyzed using GeneMapper® v 4.0 software (Applied Biosystems).

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; 0.9–1.2 Result indicates ambiguous; < 0.9 indicates mycoplasma negative.

Sendai virus detection

Total RNA was extracted using RNeasy Plus Mini Kit (Qiagen). Human fibroblasts (GM05659, Coriell Institute) after transfection with Sendai virus for 4 days was used as the positive control. 1 μg of RNA was reverse transcribed into cDNA with Superscript™ III First-Strand Synthesis SuperMix kit and PCR was performed using Platinum II Hot- Start PCR Master Mix (ThermoFisher Scientific) and the amplifications were carried out using the following program: 94 °C, 2 mins; 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).

Teratoma formation assay

Patient iPSCs cultured in 6-well plates were dissociated with DPBS containing 0.5 mM EDTA, and approximately 1×10^7 dissociated cells were resuspended in 400 μl culture medium supplied with 25mM HEPES (pH 7.4) 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 and stained with hematoxylin and eosin.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.101362.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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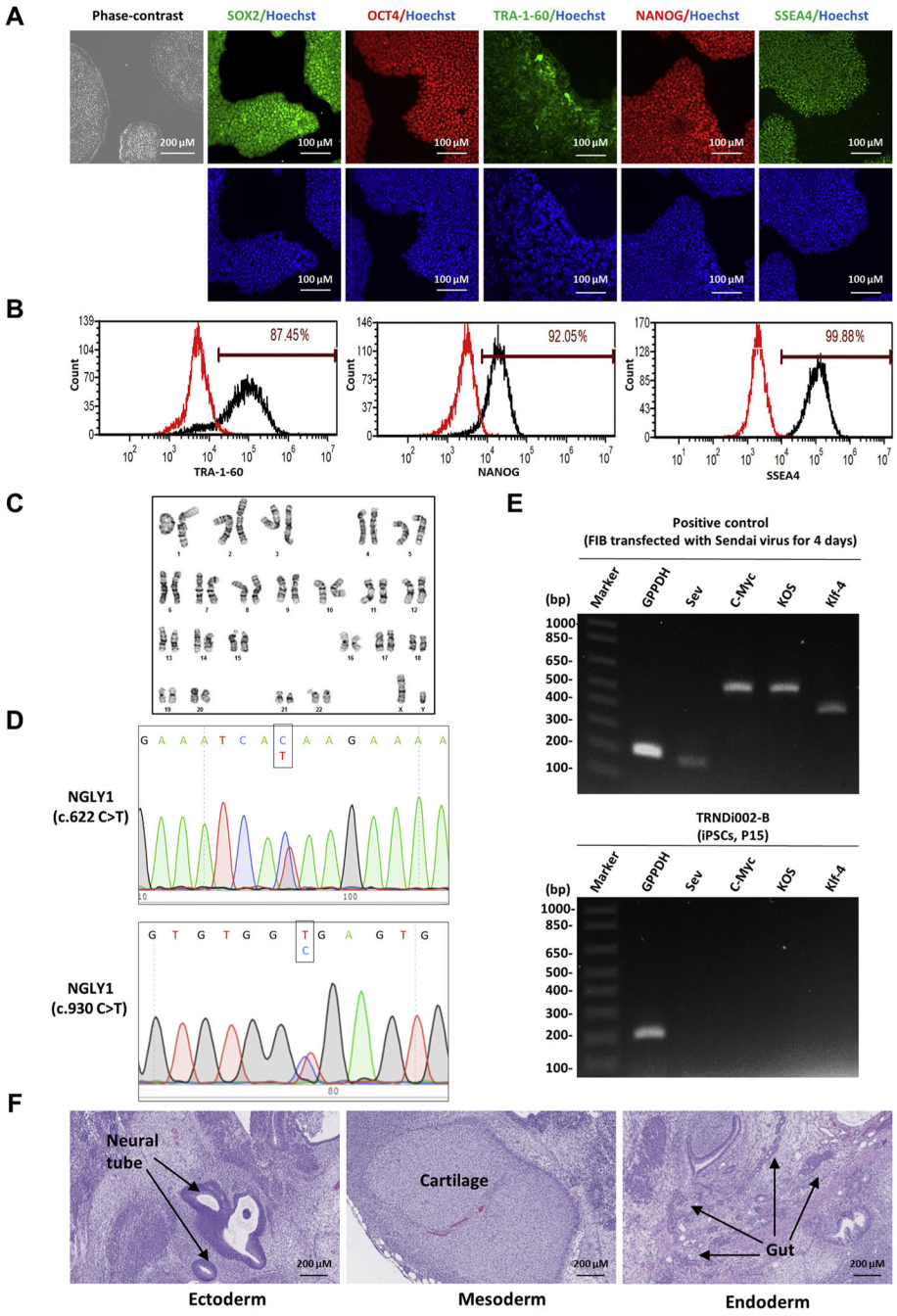

Fig. 1. Characterization of TRNDi002-B iPSC line.

A) Left: Phase contrast imaging of TRNDi002-B colonies grown on Matrigel at passage 10. Right: Representative immunofluorescent micrographs of iPSCs positive for stem cell markers: SOX2, OCT4, TRA-1-60, NANOG, and SSEA4. Nucleus is labelled with Hoechst (in 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 compound heterozygous of a p.Q208X variant (c.622C > T) in exon 4 and a p.G310G (c.930C > T) in exon 6 of the NGLY1 gene. E) RT-PCR verification of the clearance of

Sendai virus from the reprogrammed cells. Sendai virus vector transduced fibroblasts was used as positive control. **F)** Pathological analysis of a teratoma from TRNDi002-B iPSC, showing a normal ectodermal, endodermal and mesodermal differentiation.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 Panel A
Phenotype	Immunocytochemistry	SOX2, OCT4, NANOG, SSEA-4, TRA-1-60	Fig. 1 Panel A
	Flow cytometry	TRA-1-60 (87.5%); NANOG (92.1%); SSEA-4 (99.9%)	Fig. 1 Panel B
Genotype	Karyotype (G-banding) and resolution	46XY Resolution: 350–400	Fig. 1 Panel C
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	18 sites tested, all sites matched	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Compound heterozygous mutation of NGLY1	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 tube); Mesoderm (cartilage); Endoderm (gut)	Fig. 1 Panel F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
(OPTIONAL)	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, Cat# 4903, RRID: AB_10559205
Pluripotency markers	Rabbit anti-OCT4	1:400	Thermo Fisher, Cat# A13998, RRID: AB_2534182
Pluripotency markers	Mouse anti-SSEA4	1:1000	Cell signaling, Cat# 4755, RRID: AB_1264259
Pluripotency markers	Mouse anti-TRA-1-60- Alexa Fluor 488	1:10	BD Biosciences, Cat# 560173, RRID: AB_1645379
Secondary antibodies	Donkey anti-Mouse IgG (Alexa Fluor 488)	1:400	Thermo Fischer, Cat# A21202, RRID: AB_141607
Secondary antibodies	Donkey anti-Rabbit IgG (Alexa Fluor 594)	1:400	Thermo Fischer, Cat# A21207, RRID: AB_141637
Flow cytometry antibodies	Anti-Tra-1-60-DyLight 488	1:50	Thermo Fischer, 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 Fischer, Cat# 53–8843-41, RRID: AB_10597752
Flow cytometry antibodies	Mouse-IgM-DyLight 488	1:50	Thermo Fischer, Cat# MA1–194-D488, RRID: AB_2536969
Flow cytometry antibodies	Rabbit IgG-Alexa Fluor 488	1:50	Cell Signaling, Cat# 4340S, RRID: AB_10694568
Flow cytometry antibodies	Mouse IgG3-FITC	1:50	Thermo Fischer, 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 TGA TG	
House-keeping gene (RT-PCR)	GAPDH/197 bp	GGA GCG AGA TCC CTC CAA AAT/GGC TGT TGT CAT ACT TCT CAT GG	
Targeted mutation analysis (PCR)	NGLY1 (c.622C > T)/577bp	CCA TGC AGT TCA AAC CCA TGT TCT TC/GAT GCA TAG ATC AGA GCT CTG TAT TGA TCC	
Targeted mutation analysis (PCR)	NGLY1 (c.930C > T)/558bp	CTG CAA AGC CCT ACT ACC CTC/ATT AAA GGC CGG GCG CAG	