An induced pluripotent stem cell line (TRNDi010-C) from a patient carrying a homozygous p.R401X mutation in the NGLY1 gene

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Abstract

NGLY1 deficiency is a rare inherited disorder caused by mutations in the NGLY1 gene encoding N-glycanase 1 that is a hydrolase for N-linked glycosylated proteins. An induced pluripotent stem cell (iPSC) line was generated from the dermal fibroblasts of a 16-year-old patient with homozygous mutation of p.R401X (c.1201 A \textgt T) in the NGLY1 gene. Our iPSC model offers a useful resource to study the disease pathophysiology and to develop therapeutics for treatment of NGLY1 patients.

Resource utility

This TRNDi010-C iPSC line presents a patient-specific disease model for studies of NGLY1 deficiency phenotype and pathophysiology and can be used as a cell-based model for drug discovery and therapeutic development to treat NGLY1 patients.

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Declaration of Competing Interests
No potential conflicts of interest were disclosed.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101496.
NGLY1 deficiency, also known as NGLY1-related congenital disorder of deglycosylation, is a rare autosomal recessive disorder caused by mutations in the NGLY1 gene which encodes a specialized enzyme called N-glycanase that removes N-linked glycan from glycosylated proteins within the body. Deficiency of this protein can lead to malfunctions of cellular functions and accumulation of misfolded proteins within cells in specific tissues or organs. The symptoms and severity of this disease can dramatically vary among affected individuals, who may have developmental delays, intellectual disability, movement disorders, seizures, liver disease, and alacrima (Enns et al., 2014; Lam et al., 2017; Caglayan et al., 2015).

In this study, a human dermal fibroblast was derived from a 16-year-old female patient (GM26612, Coriell Institute) with a homozygous nonsense mutation of p.R401X (c.1201A > T) in exon 8 of the NGLY1 gene (3p24.2) (Enns et al., 2014; Need et al., 2012). The iPSC line, TRNDi010-C, was reprogrammed from the fibroblasts using the non-integrating CytoTune-Sendai viral vector kit (A16517, Thermo Fisher Scientific) containing four pluripotency transcription factors, OCT3/4, KLF4, SOX2, and c-MYC (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 TRNDi010-C iPSCs displayed the standard pluripotent 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). The quantitative analysis by flow cytometry revealed 98.36% and 84.09% expression rate of TRA-1–60 and NANOG, respectively (Fig. 1B). G-banded karyotyping analysis was used to confirm the karyotype, which showed the normal diploid 46, XX, without any detectable abnormalities (Fig. 1C). The genetic mutation, c.1201A > T (p.R401X), was validated by Sanger sequencing of the PCR product harboring the single nucleotide variant (Fig. 1D), consistent with the description of Coriell Institute. After passage 30, the exogenous reprogramming factors were eliminated from TRNDi010-C iPSCs, despite the remaining low level of SeV (Fig. 1E). To further test the pluripotency of this iPSC line, teratoma formation experiment was performed. As shown in Fig. 1F, the imaging data identified its ability to generate derivative of three germ layers, ectoderm, mesoderm and endoderm in vivo. Furthermore, this iPSC was negative for mycoplasma contamination (Supplementary Fig. S1). The STR DNA profile of the TRNDi010-C matched with its parental GM26612 fibroblast at all 18 loci (information available with the authors).

Materials and methods

Cell culture and reprogramming

Patient skin fibroblasts were obtained from Coriell Cell Repositories (GM26612), 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% CO2 at 37 °C. Patient fibroblasts were reprogrammed into iPSCs using the non-integrating Sendai virus technology (Beers et al., 2015). Human iPSCs were cultured in mTeSR™1 (STEMCELL Technologies) on Matrigel (Corning, 354277)-coated plates at 37 °C in humidified air with 5% CO2 and 5%
The cells were passaged with ReLeSR™ (STEMCELL Technologies) at generally 1:10 ratio when they reached around 70% confluency.

**Genome analysis**

The genome analysis of variants in NGLY1 was conducted through Applied StemCell (Milpitas, California, USA). Briefly, genomic DNA was extracted from iPSC line TRNDi010-C 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 min; 35 cycles of [95 °C, 15 s; 60 °C, 15 s; 72 °C, elongation duration varies by amplicon size], 72 °C 5 min; 4 °C, indefinite. Genotyping of the homozygous for the p.R401X variant (c.1201 A > T) in exon 8 of the NGLY1 gene was performed using Sanger sequencing analysis. The specific primers for gene amplification and sequencing are listed in Table 2.

**Immunocytochemistry**

iPSC colonies, cultured in the 96-well plate, were washed with Dulbecco’s phosphate-buffered saline (DPBS) without Ca\(^{2+}\) and Mg\(^{2+}\) and fixed in 4% paraformaldehyde for 15 min at room temperature. Fixed cells were washed with DPBS twice, and permeabilized with 0.1% Triton X-100 in DPBS for 15 min. After 1 h of blocking, the cells were incubated with primary antibodies, diluted in the blocking buffer, for overnight at 4 °C. Cells were washed twice with DPBS and a corresponding secondary antibody conjugated with Alexa Fluor 488 or Alex Fluor 647 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 min and imaged using an INCell Analyzer 2500 imaging system (GE Healthcare) with Cy5, FITC and DAPI filter sets.

**Flow cytometry analysis**

The iPSCs were dissociated by TrypLE Express enzyme (Thermo Fisher Scientific). After washing once with DPBS, cells were fixed with 4% paraformaldehyde for 10 min and were permeabilized 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 h at 4 °C. The cells were then analyzed on a BD AccuriC6 FlowCytometry system (BD Biosciences).

**G-banded karyotyping**

The G-banded karyotyping analysis was performed by the WiCell Research Institute (Madison, WI) using the iPSC cells at passage 6. Twenty randomly selected metaphase cells were selected for the standard cytogenetic analysis.

**Short tandem repeat (STR) analysis**

The STR analysis of patient fibroblasts and iPSCs was performed by the Johns Hopkins University Genetic Resources Core Facility using the Promega PowerPlex 18D Kit. The ABI Prism® 3730xl Genetic Analyzer was used to electrophorese the PCR products and GeneMapper® v 4.0 software (Applied Biosystems) was used to analyze the data.
**Mycoplasma detection**

The Lonza MycoAlert kit was used to assess the mycoplasma according to the instructions from the company. B/A ratio > 1.2 indicates the positive sample; 0.9–1.2 indicates the ambiguous result; < 0.9 indicates the negative sample.

**Sendai virus detection**

Using the RNeasy Plus Mini Kit (Qiagen), the total RNA was extracted. The cDNA was reverse-transcribed from 1 μg RNA by SuperScript™ III First-Strand Synthesis SuperMix (Thermo Fisher Scientific). The Platinum II Hot-Start PCR Master Mix (Thermo Fisher Scientific) was used to amplify the target sequence with a PCR 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 specific primers (Table 2). The human fibroblasts (GM05659, Coriell Institute) transfected with Sendai virus for 4 days was used as the positive control.

**Teratoma formation assay**

Patient iPSCs were dissociated with 0.5 mM EDTA in PBS and were resuspended approximately 1 × 10⁷ cells 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 (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 Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgment**

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Fig. 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 (98.36%); NANOG (84.09%)           | Fig. 1 Panel B            |
| Genotype                              | Karyotype (G-banding) and resolution | 46XX 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                  | Homozygous mutation NGLY1, p.R401X          | 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, 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 1
Table 2

Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | Dilution | Company Cat # and RRID |
|--------------------------------------------------------|----------|------------------------|
| Pluripotency Markers                                   |          |                        |
| Mouse anti-SOX2                                        | 1:50     | R & D systems, Cat# MAB2018, RRID: AB_358009 |
| Rabbit anti-NANOG                                      | 1:400    | Cell signaling, Cat# 4903, RRID: AB_10559205 |
| Rabbit anti-OCT4                                       | 1:400    | Thermo Fisher, Cat# A13998, RRID: AB_2534182 |
| Mouse anti-SSEA4                                       | 1:1000   | Cell signaling, Cat# 4755, RRID: AB_1264259 |
| Mouse anti-TRA-1–60                                    | 1:1000   | Cell signaling, Cat# 4746, RRID: AB_219059 |
| Secondary Antibodies                                   |          |                        |
| Donkey anti-Mouse IgG (Alexa Fluor 488)                | 1:400    | Thermo Fischer, Cat# A21202, RRID: AB_141607 |
| 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 |
| Anti-Nanog-Alexa Fluor 488                             | 1:50     | Millipore, Cat# FCABS352A4, RRID: AB_10807973 |
| Mouse-IgM-DyLight 488                                   | 1:50     | Thermo Fischer, Cat# MA1-194-D488, RRID: AB_2536969 |
| Rabbit IgG-Alexa Fluor 488                              | 1:50     | Cell Signaling, Cat# 43405, RRID: AB_10694568 |

| Primers                                               | Target   | Forward/Reverse primer (5′–3′) |
|-------------------------------------------------------|----------|--------------------------------|
| Sev specific primers (RT-PCR)                         | Sev/181 bp| F: GGA TCA CTA GGT GAT ATC GAG C |
|                                                       |          | R: ACC AGA CAA GAG TTT AAG AGA TAT GTA TC |
| KOS/528 bp                                            | F: ATG CA C CGC TAC GAC GTG AGC GC |
|                                                       | R: ACC TTG ACA ATC CTG ATG TGG |
| Klf/4410 bp                                           | F: TTC CTG CAT GCC AGA GGA GCC C |
|                                                       | R: AAT GTA TCG AAG GTG CTC AA |
| C-Myc/523 bp                                          | F: TAA CTG ACT AGC AGG CTT GTG TC G |
|                                                       | R: TCC ACA TAC AGT CCT GGA TGA TGA TG |
| House-Keeping gene (RT-PCR)                           | GAPDH/197bp| F: GGA GCG AGA TCC CTC CAA AAT |
|                                                       | R: GGC TGT TGT CAT ACT TCT CAT GG |
| Targeted mutation analysis (PCR)                       | NGLY1 (c.1201A > T)/258bp| F: GAC AAC AGA CGC AGA CTT C |
|                                                       | R: AAA AAG ATC GCC ACA CCA TAC C |
# Key resources table

| Unique stem cell line identifier | TRNDi010-C |
|----------------------------------|------------|
| Alternative name(s) of stem cell line | HT592C |
| 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: 16-year-old  
Sex: Female  
Ethnicity: Caucasian |
| Cell Source | Skin fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Integration-free Sendai viral vectors |
| Genetic Modification | Yes |
| Type of Modification | Hereditary |
| Associated disease | NGLY1 Deficiency |
| Gene/locus | NGLY1R401X |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | 04–23–2018 |
| 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 |