HHS Public Access

Author manuscript

Stem Cell Res. Author manuscript; available in PMC 2021 July 23.

Published in final edited form as:

Stem Cell Res. 2021 July ; 54: 102447. doi:10.1016/j.scr.2021.102447.

Generation of an induced pluripotent stem cell line (TRNDi031-A) from a patient with Alagille syndrome type 1 carrying a heterozygous p. C312X (c. 936 T > A) mutation in *JAGGED-1*

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Abstract

Alagille syndrome (ALGS) is a rare autosomal dominant disorder caused by disruption of the Notch signaling pathway due to mutations in either *JAGGED1 (JAG1)* (ALGS type 1) or *NOTCH2* (ALGS type 2). Loss of this signaling interferes with the development of many organs, but especially the liver. A human induced pluripotent stem cell (iPSC) line was generated from the fibroblasts of a patient with a p. C312X (c. 936 T > A) variant in *JAG1*. This iPSC line offers a valuable resource to study the disease pathophysiology and develop therapeutics to treat patients with ALGS.

1. Resource Table:

Unique stem cell line identifier	TRNDi031-A
Alternative name(s) of stem cell line	HT824A; NCATS-CL7739
Institution	National Institutes of Health National Center for Advancing Translational Sciences Bethesda, Maryland, USA

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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.2021.102447.

Unique stem cell line identifier	TRNDi031-A
Contact information of distributor	Dr. Wei Zheng, wzheng@mail.nih.gov
Type of cell line	iPSC
Origin	Human
Additional origin info requiredfor human ESC or iPSC	Age: 17 years Sex: Female Ethnicity: Caucasian
Cell Source	Fibroblasts
Clonality	Clonal
Associated disease	Alagille syndrome type 1 (JAG-Associated); Alagille-Watson syndrome; arteriohepatic dysplasia
Gene/locus	Gene: *JAG1* Locus: 20p12.2 Mutation: p. C312X (c. 936 T > *A*)
Date archived/stock date	2020
Cell line repository/bank	https://hpscreg.eu/cell-line/TRNDi031-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.

2. Resource utility

This human-induced pluripotent stem cell (iPSC) line with an ALGS type 1 mutation is a patient-specific disease model for studying the more prevalent JAG-associated ALGS phenotypes and pathophysiology. This resource can be used as a cell-based model for drug discovery and therapeutic development to treat patients with ALGS.

3. Resource details

Alagille Syndrome (ALGS) is a rare multisystem disorder caused by defects in the NOTCH signaling pathway due to mutations in either *JAGGED-1* (type 1) or, less frequently, *NOTCH2* (type 2) encoding for respectively the ligand or receptor (Turnpenny and Ellard, 2012). NOTCH is a core intercellular signaling pathway in the embryonic development of the organ systems affected by ALGS. Common clinical characteristics of ALGS are peripheral pulmonary stenosis, butterfly vertebrae, cholestasis due to bile duct paucity, and posterior embryotoxon (Mitchell et al., 2018). ALGS has high variable expressivity causing clinical features to be disparate among affected patients (Turnpenny and Ellard, 2012). There are currently no approved therapeutics for ALGS, and treatment is limited to symptom management by a multidisciplinary medical team. ALGS is a leading cause of childhood liver transplants as less than 25% of patients reach age 19 with their natural liver (Kamath et al., 2020). Maralixibat has been studied for pruritus associated with ALGS. Following a potential clearance of a New Drug Application in late 2021, it could become the first FDA-approved treatment available for ALGS (Shneider et al., 2018).

This study has established a human iPSC line TRNDi031-A from the fibroblasts of a female patient (GM11091, Coriell Institute) carrying a heterozygous mutation, p. C312X (c. 936 T > A) which creates a premature stop codon in the seventh exon of *JAG1* (Table 1, Fig. 1D). The ALGS 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, TRNDi031-A, exhibited a 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 expression rates of pluripotency markers, TRA-1–60 (87%), NANOG (94%), and SSEA-4 (100%), confirming these pluripotency markers (Fig. 1B). G-banded karyotype analysis confirmed a normal karyotype (46, XX) with no detectable abnormalities (Fig. 1C). The genetic mutation was identified 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 SeVspecific primers, and the vectors were eliminated by passage 15 (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 TRNDi031-A matched with its parental GM11091 fibroblast at 16 loci.

4. Materials and methods

4.1. Cell culture and reprogramming

Patient fibroblasts (GM11091) were obtained from Coriell Cell Repositories 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. 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% CO_2 and 5% O_2. The cells were passaged with EZ-LiFT™ (Sigma-Aldrich) at a generally 1:6 ratio when they reached 70% confluency with 10 μM ROCK inhibitor.

4.2. Genome analysis

The gene analysis was conducted at passage 5 through Applied StemCell (Milpitas, California, USA). Genomic DNA was extracted from the iPSC 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.

4.3. Immunocytochemistry

Patient iPSCs cultivated at passage 20 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 and 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 33,342 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.

4.4. Flow Cytometry analysis

The iPSCs at passage 17 were dissociated, washed once with DPBS, and fixed with 4% paraformaldehyde 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 h at 4 °C. The cells were analyzed with a BD Accuri™ C6 Flowcytometry system (BD Biosciences).

4.5. G-banding karyotype

The G-banded karyotyping analysis was performed at passage 11 by WiCell Research Institute (Madison, WI). Twenty randomly selected metaphase cells were used for the standard cytogenetic analysis.

4.6. Short tandem repeat (STR) DNA profile analysis

STR analyses of patient fibroblasts and derived iPSCs at passage seven were performed by WiCell Research Institute using a PowerPlex® 16 HS System.

4.7. Mycoplasma detection

The Lonza MycoAlert kit was used according to the instructions on cells at passage 20. B/A ratio > 1.2 indicates the positive sample; 0.9–1.2 indicates the ambiguous result; less than 0.9 indicates the negative sample.

4.8. Sendai virus detection

Total RNA of derived iPSCs at passage 15 was extracted using RNeasy Plus Mini Kit (Qiagen), and 0.5 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 mins; 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 (GM0559, Coriell Institute) transfected with Sendai virus for four days were used as a positive control.

4.9. Teratoma formation assay

Patient iPSCs at passage nine 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). Afterward, 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 post-injection 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.

Acknowledgment

This work was supported by the Intramural Research Programs of the National Center for Advancing Translational Sciences, National Institutes of Health, and was a CRADA collaboration between NCATS, The Alagille Syndrome Alliance (ALGSA), and Travere Therapeutics.

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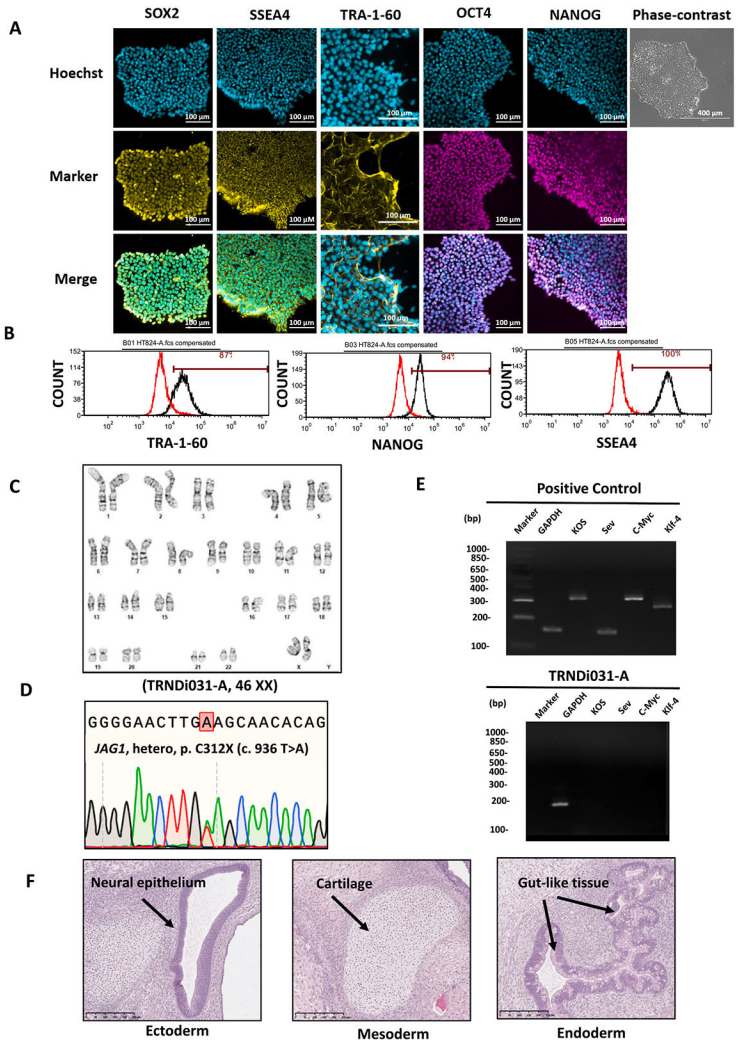

Fig. 1.

Characterization of TRNDi031-AiPSC line. (A) Left: Immunofluorescence images of iPSCs positive for stem cell markers: SOX2, SSEA4, TRA-1-60, OCT4, and NANOG. Nucleus is labelled with Hoechst 33342 (blue). Right: Phase contrast image of TRNDi031-A colonies. (B) Flow cytometry analysis of pluripotency protein markers: TRA-1-60, NANOG and SSEA4. (C) Cytogenetic analysis showing a normal karyotype (46, XX). (D) Detection of heterozygous gene mutation of c.617G>A in the *ACVR1* gene. (E) RT-PCR confirmation for the clearance of the Sendai virus from reprogrammed cells. Sendai virus vector transduced fibroblasts was used as a positive control. (F) Pathological analysis of teratoma from TRNDi031-AiPSC showing a normal ectodermal, mesodermal, and endodermal differentiation.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Figure 1 panel A
Phenotype	Qualitative analysisImmunocytochemistry	SOX2, OCT4, NANOG, SSEA-4, TRA-1-60	Figure 1 panel A
	Quantitative analysisFlow cytometry	TRA-1-60 (87%); NANOG (94%); SSEA-4 (100%)	Figure 1 panel B
Genotype	Karyotype (G-banding) and resolution	46XXResolution: 475–525	Figure 1 panel C
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not performed	N/A
		15 sites tested; all sites matched	Submitted in an archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous of *JAG1*, p. C312X (c. 936 T > A)	Figure 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)	Figure 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
Genotype additional info (OPTIONAL)	HLA tissue typing	N/A	N/A

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	AB_358009
Pluripotency Markers	Rabbit anti-NANOG	1:400	Cell Signaling Technology, Cat # 4903	AB_10559205
Pluripotency Markers	Rabbit anti-OCT4	1:400	Thermo Fisher Scientific, Cat # A13998	AB_2534182
Pluripotency Markers	Mouse anti-SSEA4	1:500	Cell Signaling Technology, Cat # 4755	AB_1264259
Pluripotency Markers	Mouse anti-TRA-1–60	1:500	Cell Signaling Technology, Cat # 4746	AB_1264259
Secondary Antibodies	Donkey anti-Mouse IgG (Alexa Fluor 488)	1:400	Thermo Fisher Scientific, Cat # A21202	AB_141607
Secondary Antibodies	Donkey anti-Rabbit IgG (Alexa Fluor 594)	1:400	Thermo Fisher Scientific, Cat # 21,207	AB_141637
Flow Cytometry Antibodies	Anti-TRA-1-60-DyLight488	1:50	Thermo Fisher Scientific, Cat # MA1-023-D488X	AB_2536700
Flow Cytometry Antibodies	Anti-Nanog-Alexa Fluor 488	1:50	Sigma-Aldrich, Cat # FCABS3524	AB_10807973
Flow Cytometry Antibodies	Anti-SSEA-4-Alexa Fluor 488	1:50	Thermo Fisher Scientific, Cat # 53–8843-41	AB_10597752
Flow Cytometry Antibodies	Mouse IgG3-FITC	1:50	Thermo Fisher Scientific, Cat# 11–4742-42	AB_2043894
Flow Cytometry Antibodies	Rabbit IgG-Alexa Fluor 488	1:50	Cell Signaling Technologies, Cat # 4340S	AB_10694568
Flow Cytometry Antibodies	Mouse-IgM-DyLight 488	1:50	Thermo Fisher Scientific, Cat # MA1-194-D488	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)	*JAG1*	991 bp	TCC TTT TGT CAG GAG TCG GC/GCG TGT GAT AGA ACC CTG CT	