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Lab Resource: Single Cell Line

Generation of Alagille syndrome derived induced pluripotent stem cell line carrying heterozygous mutation in the JAGGED-1 gene at splicing site (Chr20: 10,629,709C>A) before exon 11

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A B S T R A C T

Alagille syndrome (ALGS) is a multisystem autosomal dominant disorder caused by defects in the Notch signaling pathway, including the mutation in *JAGGED1* (*JAG1*) (ALGS type 1) or *NOTCH2* (ALGS type 2). An induced pluripotent stem cell (iPSC) line was generated from the dermal fibroblasts of a 3-month-old patient with heterozygous mutation at *JAG1* splicing site (Chr20: 10,629,709C>A) before exon 11. This iPSC model offers a useful resource for disease modeling to study the disease pathophysiology and to develop therapeutics for treatment of ALGS.

Resource Table

Unique stem cell line identifier	TRNDi029-A
Alternative name(s) of stem cell line	NCATS-CL4870, HT822A
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: 3-month-old Sex: Female Ethnicity if known:
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Integration-free Sendai viral vectors
Genetic Modification	NO
Type of Modification	N/A
Associated disease	Alagille syndrome
Gene/locus	*JAGGED-1* gene at splicing site (Chr20: 10,629,709C>A) before exon 11, genome reference: GRCh37
Method of modification	N/A N/A

(*continued on next column*)

Resource Table (*continued*)

Name of transgene or resistance	
Inducible/constitutive system	N/A
Date archived/stock date	February 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/TRNDi029-A
Ethical approval	Coriell Institute for Medical Research harvested the fibroblasts from patient and their 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.

1. Resource utility

This iPSC line presents a patient-specific disease model for studies of ALGS deficiency phenotype and pathophysiology and can serve as a cell-based model for therapeutics development to treat ALGS patients.

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2. Resource details

Alagille syndrome (ALGS), also known as arteriohepatic dysplasia, is an autosomal dominant, multisystem disorder with variable phenotypic penetrance. ALGS is caused by heterozygous mutations in one of two genes, *JAGGED1* (*JAG1*) (more than 90%) and *NOTCH2*, that are fundamental components in the Notch signalling pathway. Traditionally, the clinical diagnosis of ALGS was based on at least three of the clinical features: intrahepatic bile duct paucity causing chronic cholestasis, cardiac defect, ophthalmologic abnormalities, skeletal abnormalities, and characteristic facial features Mitchell et al. (2018). In addition, the renal and vascular diseases are also highly prevalent among ALGS patients. Of note, in the same family, the identical genetic mutation often leads to distinct phenotypic characteristics [1]. The relationship between genotype and phenotype remains yet to be investigated.

In this study, a human induced pluripotent stem cell (iPSC) line was established from fibroblasts of a 3-month-old female patient (GM05759, Coriell Institute) carrying heterozygous mutation of the *JAG1* gene at splicing site (Chr20: 10,629,709C>A, genome reference: GRCh37) before exon 11 (Table 1). Non-integrating Sendai virus (SeV) vectors encoding OCT3/4, KLF4, SOX2, and c-MYC were employed to reprogram the patient's fibroblasts into iPSCs. The generated iPSC line named NCATS-CL4870 (or TRNDi029-A) was confirmed by a classical embryonic stem cell morphology and positive expression of SOX2, OCT4, TRA-1-60, NANOG, and SSEA4 (Fig. 1A). The quantitative analysis by flow cytometry showed 96.6% and 97.3% expression rates of TRA-1-60 and NANOG, respectively (Fig. 1B). The normal karyotype (46, XX) was confirmed by the G-banded karyotyping (Fig. 1C). The genetic mutation of the *JAG1* gene at splicing site (Chr20: 10,629,709C>A) before exon 11 (Table 1) was validated by Sanger sequencing of the PCR product harbouring the single nucleotide variant (Fig. 1D), that is consistent with the description of original mutation by Coriell Institute. After passage 15, the clearance of SeV vectors was confirmed with reverse transcription polymerase chain reaction (RT-PCR) using SeV-specific primers (Fig. 1E). In the teratoma formation test, the iPSC line was capable of differentiating into the three germ layers, ectoderm, mesoderm, and endoderm, in immunocompromised mice (Fig. 1F). Furthermore, this iPSC line was not contaminated with mycoplasma (Supplementary Fig. S1)****, and STR DNA profiling analysis demonstrated the matching genotypes at all 16 loci examined (information available with the authors).

3. Materials and methods

Cell culture and reprogramming

Patient skin fibroblasts were obtained from Coriell Cell Repositories (GM05759), 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. The reprogramming was conducted by using the non-integrating Sendai virus technology Beers et al. (2015). Generated human iPSCs were cultured in mTeSR™1 (STEMCELL Technologies) 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 EZ-LiFT™ (Millipore Sigma) at generally 1:10 ratio when they reached around 70% confluency.

Genome analysis

The genome analysis of mutation in *JAG1* was conducted by Applied StemCell (Milpitas, CA, USA). In brief, genomic DNA was extracted from iPSC line using QuickExtract™ DNA Extraction Solution (Lucigen) followed by PCR amplification using MyTaq™ Red Mix (Bioline, Taunton, MA). Amplifications were performed on 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, 1 min], 72 °C, 5 min; 4 °C, indefinite. Genotyping of the heterozygous mutation (*JAG1* gene at splicing site (Chr20: 10,629,709C > A) before exon 11) was conducted using Sanger sequencing analysis. The specific primers for gene amplification and sequencing are listed in Table 2.

Immunocytochemistry

iPSCs cultured at passage 15 in Matrigel-coated 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 with bocking buffer (Cell Staining Buffer, BioLegend) for 1 h. The cells were then incubated with primary antibodies, diluted in the blocking buffer, for overnight at 4 °C. Cells were washed twice with DPBS and incubated with corresponding secondary antibodies for 1 h at room temperature (antibodies used are listed in Table 2). Cell nuclei were stained with Hoechst 33,342 for 15 min and imaging was carried out by using an INCell Analyzer 2500 imaging system (GE Healthcare).

Flow cytometry analysis

The iPSCs at passage 1 were dissociated, washed once with DPBS, and then 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. Finally, the cells were analysed with a BD Accuri™ C6 FlowCytometry system (BD Biosciences).

G-banded karyotyping

The G-banded karyotyping analysis was conducted by the WiCell Research Institute (Madison, WI). A total of twenty randomly picked metaphase cells were used for the standard cytogenetic analysis.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 Panel A
Phenotype	Immunocytochemistry	SOX2, OCT4, TRA-1–60, NANOG, SSEA-4	Fig. 1 Panel A
	Flow cytometry	TRA-1–60: 96.6%; NANOG: 97.3%	Fig. 1 Panel B
Genotype	Karyotype (G-banding) and resolution	46XXResolution 400–425	Fig. 1 Panel C
Identity	Microsatellite PCR (mPCR) OR STR analysis	Not performed 16 sites tested; all sites matched	N/A Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous mutation of JAGGED-1 gene at splicing site (Chr20: 10,629,709C > A) before exon 11	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 (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

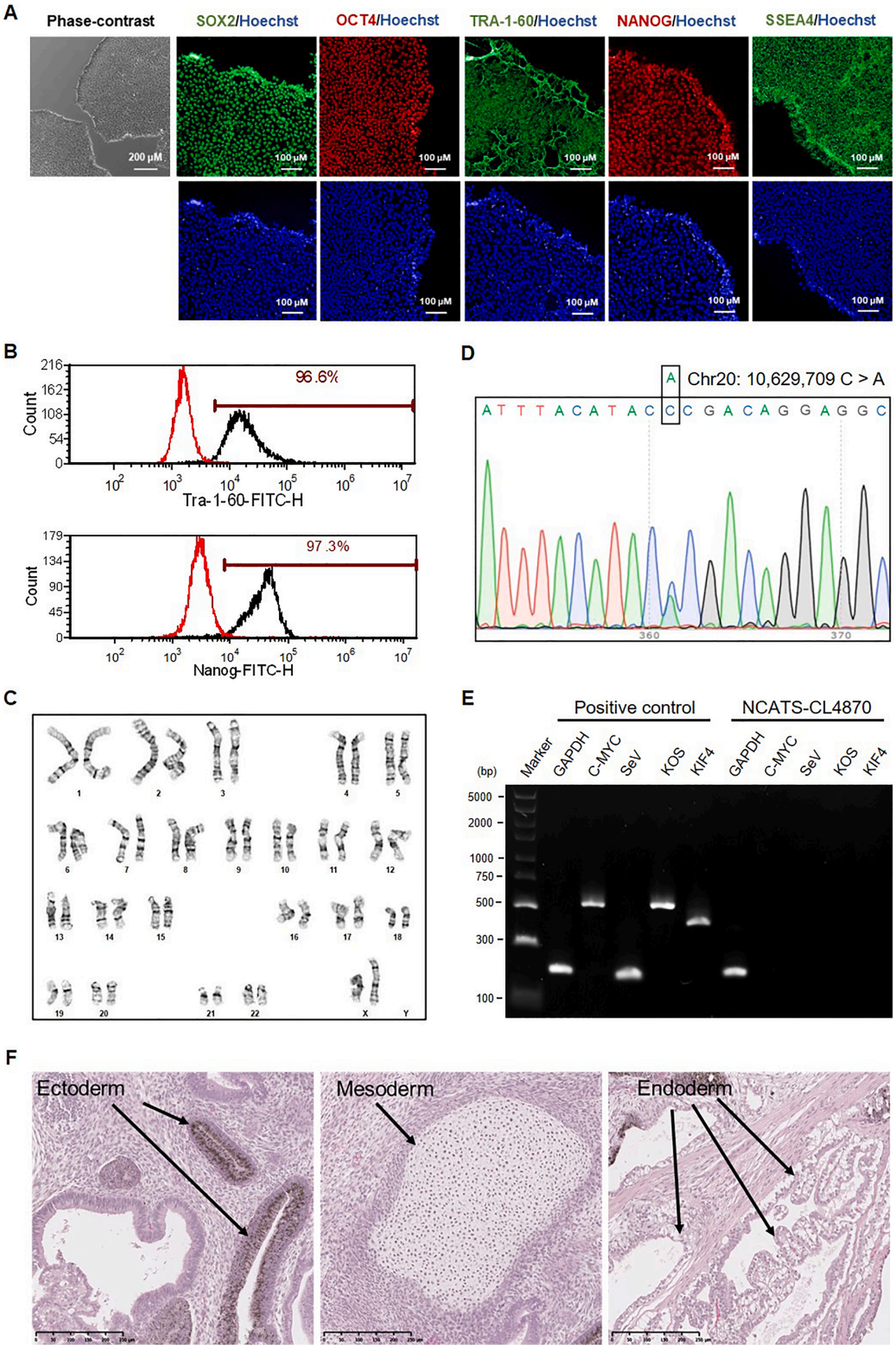
A
Phase-contrast
SOX2/Hoechst
OCT4/Hoechst
TRA-1-60/Hoechst
NANOG/Hoechst
SSEA4/Hoechst
200 μM
100 μM
B
Count
96.6%
Tra-1-60-FITC-H
97.3%
Nanog-FITC-H
C
D
A Chr20: 10,629,709 C > A
A T T T A C A T A C C C G A C A G G A G G C
360
370
E
Positive control
NCATS-CL4870
Marker
GAPDH
C-MYC
SeV
KOS
KIF4
(bp)
5000
2000
1000
750
500
300
100
F
Ectoderm
Mesoderm
Endoderm

Fig. 1.

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-OCT4	1:400	Thermo Fisher, Cat# A13998, RRID: AB_2534182
Pluripotency Markers	Mouse anti-TRA-1–60	1:500	Cell signaling, Cat# 4746, RRID: AB_2119059
Pluripotency Markers	Rabbit anti-NANOG	1:400	Cell signaling, Cat# 4903, RRID: AB_10559205
Pluripotency Markers	Mouse anti-SSEA4	1:500	Cell signaling, Cat# 4755, RRID: AB_1264259
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
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			
Sev specific primers (RT-PCR)	KOS/528 bp	F: ATG CAC CGC TAC GAC GTG AGC GC	
R: ACC TTG ACA ATC CTG ATG TGG			
Sev specific primers (RT-PCR)	Klf4/410 bp	F: TTC CTG CAT GCC AGA GGA GCC C	
R: AAT GTA TCG AAG GTG CTC AA			
Sev specific primers (RT-PCR)	C-Myc/523 bp	F: TAA CTG ACT AGC AGG CTT GTC G	
R: TCC ACA TAC AGT CCT GGA TGA TGA TG			
House-Keeping gene (RT-PCR)	GAPDH/197 bp	F: GGA GCG AGA TCC CTC CAA AAT	
R: GGC TGT TGT CAT ACT TCT CAT GG			
Targeted mutation analysis (PCR)	*JAG1* gene at splicing site (Chr20: 10,629,709C > A) before exon 11/1272 bp	F: CAG ACA AAC TCT GGC CTG TTC	
R: CAG ACA CAA GAG CTG AGG GAA			

Short tandem repeat (STR) analysis

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

Mycoplasma detection

Mycoplasma test for the cells at passage 15 was carried out using a Lonza MycoAlert kit following manufacturer's instruction. A ratio of B/A greater than 1.2 indicates mycoplasma positive; 0.9–1.2 Result indicates ambiguous; < 0.9 indicates mycoplasma negative.

Sendai virus detection

Total RNA of derived iPSCs 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 to 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.

Teratoma formation assay

Patient iPSCs at passage 15 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 added and blended 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.

Acknowledgement

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