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 \textit{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 \textit{JAGGED1} (\textit{JAG1}) (ALGS type 1) or \textit{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 \textit{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.
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 iPSC 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 SeV-specific 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% CO₂ 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% CO₂ and 5% O₂. 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.

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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 $1 \times 10^7$ 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.

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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 analysis Immunocytochemistry | SOX2, OCT4, NANOG, SSEA-4, TRA-1-60                                       | Figure 1 panel A                          |
|                                     | Quantitative analysis Flow cytometry | TRA-1-60 (87%); NANOG (94%); SSEA-4 (100%)                              | Figure 1 panel B                          |
| Genotype                            | Karyotype (G-banding) and resolution | 46XX Resolution: 475–525                                               | Figure 1 panel C                          |
| Identity                            | Microsatellite PCR (mPCR) OR STR analysis | Not performed 15 sites tested; all sites matched                           | N/A                                       |
| 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); Endoderm (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

### Antibodies used for immunocytochemistry/flow-cytometry

| Antibody                      | Dilution | Company               | Cat #              | RRID       |
|-------------------------------|----------|-----------------------|--------------------|------------|
| Pluripotency Markers          |          |                       |                    |            |
| Mouse anti-SOX2               | 1:50     | R & D systems         | MAB2018            | AB_358009  |
| Rabbit anti-NANOG             | 1:400    | Cell Signaling Technology | 4903               | AB_10559205 |
| Rabbit anti-OCT4              | 1:400    | Thermo Fisher Scientific | A13998             | AB_2534182 |
| Mouse anti-SSEA4             | 1:500    | Cell Signaling Technology | 4755               | AB_1264259 |
| Pluripotency Markers          |          |                       |                    |            |
| Mouse anti-TRA-1–60           | 1:500    | Cell Signaling Technology | 4746               | AB_1264259 |
| Secondary Antibodies          |          |                       |                    |            |
| Donkey anti-Mouse IgG (Alexa Fluor 488) | 1:400 | Thermo Fisher Scientific | A21202             | AB_141607  |
| Secondary Antibodies          |          |                       |                    |            |
| Donkey anti-Rabbit IgG (Alexa Fluor 594) | 1:400 | Thermo Fisher Scientific | 21,207             | AB_141637  |
| Flow Cytometry Antibodies     |          |                       |                    |            |
| Anti-TRA-1-60-DyLight488      | 1:50     | Thermo Fisher Scientific | MA1-023-D488X      | AB_2536700 |
| Flow Cytometry Antibodies     |          |                       |                    |            |
| Anti-Nanog-Alexa Fluor 488    | 1:50     | Sigma-Aldrich         | FCABS3524          | AB_10807973 |
| Flow Cytometry Antibodies     |          |                       |                    |            |
| Anti-SSEA-4-Alexa Fluor 488   | 1:50     | Thermo Fisher Scientific | 53–8843-41         | AB_10597752 |
| Flow Cytometry Antibodies     |          |                       |                    |            |
| Mouse IgG3-FITC               | 1:50     | Thermo Fisher Scientific | 11–4742–42         | AB_2043894 |
| Flow Cytometry Antibodies     |          |                       |                    |            |
| Rabbit IgG-Alexa Fluor 488    | 1:50     | Cell Signaling Technologies | 4340S              | AB_10694568 |
| Flow Cytometry Antibodies     |          |                       |                    |            |
| Mouse-IgM-DyLight 488         | 1:50     | Thermo Fisher Scientific | MA1-194-D488       | AB_2536969 |

### Primers

| Target                        | Size of band | Forward/Reverse primer (5’-3’)                                                                 |
|-------------------------------|--------------|---------------------------------------------------------------------------------------------|
| SeV specific primers (RT-PCR) | 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) | 528 bp       | ATG CAC CGC TAC GTG AGC GC/ACC TTG ACA ATC CTG ATG TGG                                       |
| SeV specific primers (RT-PCR) | 410 bp       | TTC CTG CAT GCC AGA GGA GCC C/AAT GTA TCG AAG GTG CTC AA                                      |
| SeV specific primers (RT-PCR) | 523 bp       | TAA CTG ACT AGC AGG CTT GTT G/TCC ACA TAC AGT CCT GGA TGA TG                                 |
| House-Keeping gene (RT-PCR)   | 197 bp       | GGA GCG AGA TCC CTC CAA AAT/GGC TGT CAT ACT TCT CAT GG                                       |
| Targeted Mutation analysis (PCR) | 991 bp     | TCC TTT TGT CAG GAG TCG GC/GCG TGT GAT AGA ACC CTG CT                                        |