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Lab resource: Stem Cell Line

Establishment of a human induced pluripotent stem cell line (SDQLCHi037-A) from a patient with Alagille syndrome carrying heterozygous mutation in JAG1 gene

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A B S T R A C T

Alagille syndrome is a complex multisystem autosomal dominant disorder that is caused by a defect in the Notch signaling pathway. We established an induced pluripotent stem cell (iPSC) line from peripheral blood mononuclear cells of a 3-month-old boy with Alagille syndrome carrying a heterozygous mutation c.1615C > T (p.Q539X) in JAG1 gene. This iPSC line was free of exogenous gene, expressed pluripotency markers, had normal karyotype, exhibited differentiation potential and harbored the same mutations found in the patient. This iPSC line offers a cell-based model for drug screening studies.

1. Resource table

Unique stem cell line identifier	SDQLCHi037-A
Alternative name(s) of stem cell line	N/A
Institution	Research Institute of Pediatrics, Qilu Children's Hospital of Shandong University, Jinan, China
Contact information of distributor	Yi Liu, y_liu99@sina.com
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 3 months old Sex: male
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Transgene free (episomal vectors)
Genetic Modification	Yes
Type of Modification	Hereditary
Associated disease	Alagille syndrome
Gene/locus	Mutation: JAG1/ chr20:10628713, mutation: c.1615C > T
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	December 2020
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/SDQLCHi037-A
Ethical approval	The study was approved by Medical Ethics Committee of Qilu Children's Hospital of Shandong University, Approval number: ETYY-2019203

2. Resource utility

Alagille syndrome is a pediatric multisystem disease characterized by various degrees of abnormalities in multiple organs, most commonly due to heterozygous mutation in the JAG1 gene (Micaglio et al., 2019). The patient-specific iPS cells will be useful to study pathogenic mechanisms and gene therapy of the disease.

3. Resource details

Alagille syndrome (OMIM 118450), also referred to as Alagille-Watson syndrome, is an autosomal dominant disorder associated with abnormalities of the heart, liver, skeleton, eye and a characteristic facial appearance. Most cases are associated with mutations in JAGGED1 (JAG1), which encodes a Notch ligand, although it is not clear how these contribute to disease development (Andersson et al., 2018). In this study, we established an iPSC line from a 3-month-old boy with Alagille

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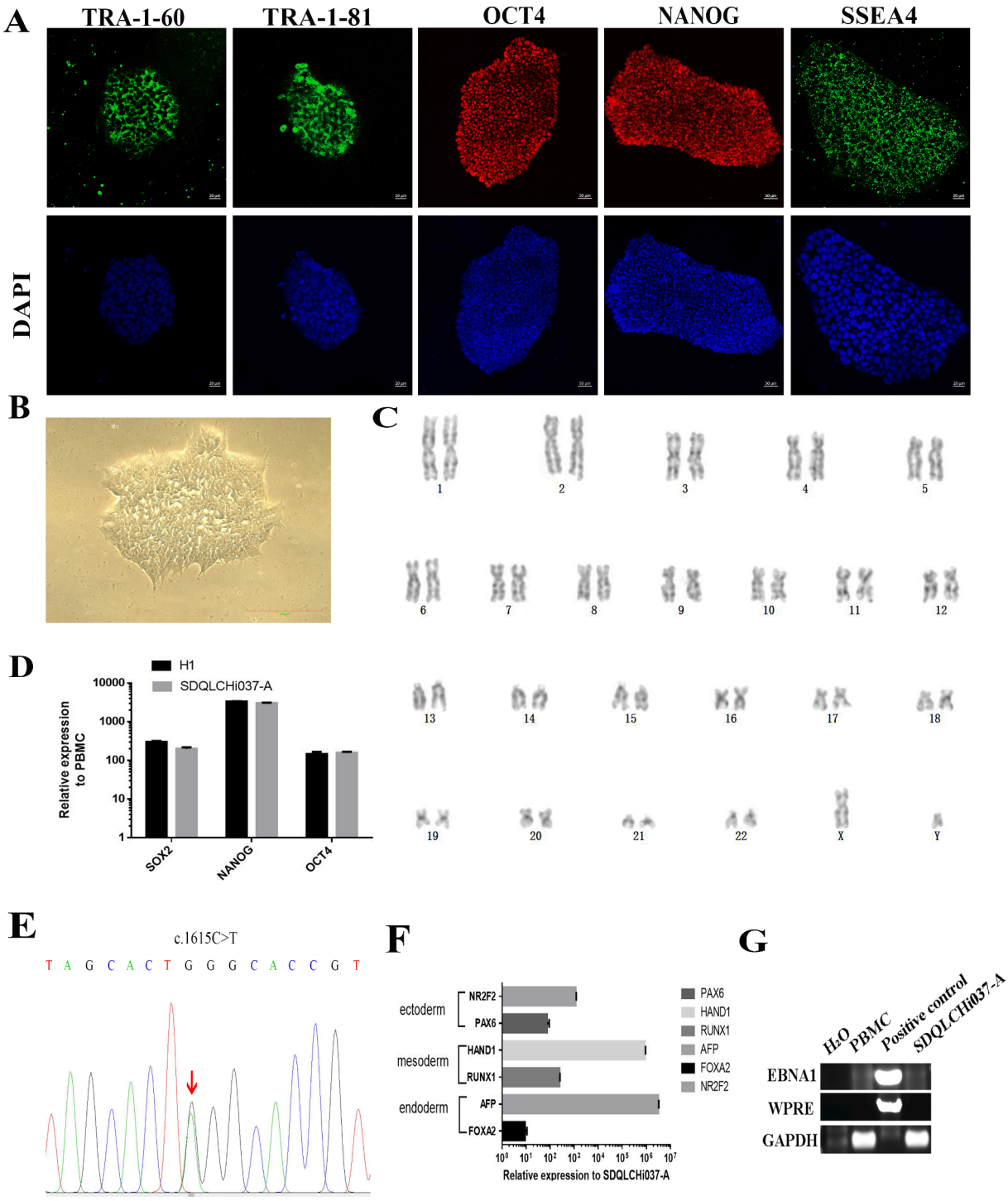

Fig. 1. Characterization and validation.

syndrome carrying heterozygous mutation c.1615C > T (p.Q539X) in JAG1 gene.

The patient-specific iPSCs were established with non-integrating episomal vectors using peripheral blood mononuclear cells (PBMCs) isolated from the patient with Alagille-Watson syndrome. SDQLCHi037-A colonies presented distinct colony border and high nuclear to cytoplasmic ratios which is the typical morphology of human embryonic stem cell (hESC) (Fig. 1B). The expression of pluripotent markers was examined by immunocytochemical staining using antibodies against human TRA-1-60, TRA-1-81, SSEA4, OCT4 and NANOG (Fig. 1A). Quantitative reverse transcription real time PCR (qRT-PCR) further supported the activation of endogenous pluripotent genes such as OCT4, SOX2 and NANOG (Fig. 1D). The karyotype of SDQLCHi037-A iPSC line revealed a normal 46, XY karyotype at passage 11 (Fig. 1C). Our iPSCs kept the original heterozygous mutation c.1615C > T (p.Q539X) in JAG1 gene which was confirmed by Sanger sequencing (Fig. 1E). Trilineage differentiation potential was detected in vitro and confirmed by the expression of endoderm (AFP/FOXA2), ectoderm (PAX6 /NR2F2) and mesoderm (HAND1/RUNX1) markers (Fig. 1F). The exogenous episomal vectors were cleared after about 11 passages (Fig. 1G). STR analysis data proved genetic match between the SDQLCHi037-A cells and the original PBMCs (STR data available with the authors).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel B
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining for TRA-1-60, OCT4, TRA-1-81, SSEA4 and NANOG	Fig. 1 panel A
	Quantitative analysis qRT-PCR	Expression of endogenous pluripotent markers SOX2, OCT4 and NANOG	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46XX, Resolution 400	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	20 loci analyzed, all matching	available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	homozygous mutant in JAG1 gene	Fig. 1 panel E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by Hoechst staining: negative.	supplementary material 1
Differentiation potential	Embryoid body formation	Expression of lineage markers: endoderm (AFP/FOXA2), ectoderm (PAX6 /NR2F2) and mesoderm (HAND1/ RUNX1)	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

Furthermore, the presence of mycoplasma contamination was excluded by luminescence assay (Supplementary file). Characterization of the generated iPSC line is summarized in Table 1.

4. Materials and methods

4.1. Cell culture and reprogramming

Peripheral blood mononuclear cells (PBMCs) were isolated from 2 ml of peripheral blood collected from the patient using density gradient centrifugation with Ficoll-Hypaque (1.077 g/ml) (G&E Healthcare) at 400 g for 30 min at room temperature (RT) and cultured in erythroid medium (serum free medium (SFM) supplemented with 100 ng/ml hSCF (PeproTech), 40 ng/ml IGF-1 (Pepro Tech), 10 ng/ml IL-3 (PeproTech), 2 U/ml EPO (R&D Systems), 100 μg/ml holotransferring (R&D Systems) and 1 μM dexamethasone (Sigma-Aldrich)) for 5 or more days. 2×10^6 PBMCs were collected and electroporated with targeting vectors including pEV-SFFV-OCT4-E2A-SOX2- Wpre, pEV-SFFV-BLC-XL-Wpre, pEV-SFFV-KLF4-Wpre and pEV-SFFV-Myc-Wpre (Novobiotec, Beijing, China) using Amaxa P3 Primary Cell Nucleofector Kit following the manufacturer's instruction of 4D Nucleofector System (Lonza, program EO-100). After transfection, cells were plated onto 12-wells pre-coated with mitomycin C -inactivated mouse embryonic fibro- -blasts (MEF).

Table 2
Reagents details. RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.

Antibodies used for immunocytochemistry/flow-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit anti-OCT4	1:500	Abcam Cat# ab19857, RRID: AB_445175
Pluripotency markers	Mouse anti-SSEA-4	1:500	Cell Signaling Inc. Cat# 4755, RRID: AB_1264259
Pluripotency markers	Mouse anti-TRA-1-81	1:1000	Cell Signaling Inc. Cat# 4745, RRID: AB_2119060
Pluripotency markers	Mouse anti-TRA-1-60	1:1000	Cell Signaling Inc. Cat# 4746, RRID: AB_2119059
Pluripotency markers	Rabbit anti-Nanog	1:1000	Abcam Cat# ab21624, RRID: AB_446437
Secondary antibodies	Goat anti-rabbit IgG CoraLite594 conjugated	1:500	Proteintech Cat# SA00006–3, RRID: AB_2810984
Secondary antibodies	Goat anti-mouse IgM Alexa Fluor 488 conjugated	1:500	Abcam Cat# ab150121, RRID: AB_2801490
Secondary antibodies	Goat anti-mouse IgG CoraLite488 conjugated	1:500	Proteintech Cat# SA00013–1, RRID: AB_2810983

Primers

	Target	Forward/Reverse primer (5′-3′)
Episomal Plasmids (PCR)	*EBNA1/666 bp*	*TTTAATACGATTGAGGGCGTCT/ GGTTTTGAAGGATGCGATTAAG*
Episomal Plasmids (PCR)	*Wpre/1741 bp*	*CCTGCTTCTCGCTTCTGTTC/ AAGCCATACGGGAAGCAATA*
References for PCR	*GAPDH/152 bp*	*GTGGACCTGACCTGCCGTCT/ GGAGGAGTGGGTGTCGCTGT*
Pluripotency Markers (qRT-PCR)	*OCT4(endogenous)/ 164 bp*	*CCTCACTTCACTGCACTGTA/ CAGGTTTTCTTTCCCTAGCT*
Pluripotency Markers (qRT-PCR)	*SOX2(endogenous)/ 151 bp*	*CCCAGCAGACTTCACATGT/ CCTCCCATTTCCCTCGTTTT*
Pluripotency Markers (qRT-PCR)	*NANOG/154 bp*	*TGAACCTCAGCTACAAACAG/ TGGTGGTAGGAAGAGTAAAG*
House-Keeping Genes (qRT-PCR)	*GAPDH/152 bp*	*GTGGACCTGACCTGCCGTCT/ GGAGGAGTGGGTGTCGCTGT*
Targeted mutation analysis (Sanger sequencing)	*JAG1/449 bp*	*ACACAGGCAGGATCATTTCAC/ TTGGCAGTCCCTACAGCATAG*
Embryoid body formation (RT-PCR)	*NR2F2/ 192 bp*	*GACCAGCACCATCGCAACC/ GCGCAACAGCAGGGAAAT*
Embryoid body formation (RT-PCR)	*PAX6/ 110 bp*	*GTCCATCTTTGCTTGGGAAA/ TAGCCAGGTTGCGAAGAACT*
Embryoid body formation (RT-PCR)	*HAND1/120 bp*	*TCAAGGCTGAACTCAAGAAGG/ TGCGTCCTTTAATCCTCTTCTC*
Embryoid body formation (RT-PCR)	*RUNX1/136 bp*	*AAGCTTCACTCTGACCATCACT/ GGGCTTGGTCTGATCATCTAGT*
Embryoid body formation (RT-PCR)	*AFP/96 bp*	*ACAATTCTTCTTTGGGCTGCTC/ TGTTGCTGCCTTTGTTTGGAA*
Embryoid body formation (RT-PCR)	*FOXA2/ 122 bp*	*GGAGCGGTGAAGATGGAA/ TACGTGTTCATGCCGTTCAT*

A day later, half-changed the medium with ReproTeSR medium (Stem Cell Technologies). iPSC colonies were manually picked up at around days 16 and cultured in mTeSR1 medium (Stem Cell Technologies) at 37 °C and 5% CO2 humidified atmosphere.

4.2. Immunofluorescence

The cells were fixed with 4% paraformaldehyde for 15 min at room temperature (RT) and blocked with QuickBlock™ Blocking Buffer for Immunol Staining (Beyotime, China) after washed by PBS three times. Afterwards, cells were incubated with primary antibodies to TRA-1-60, NANOG, SSEA4, OCT4 and TRA-1-81 at 4 °C overnight and visualized with the corresponding secondary antibodies for 1 h at room temperature in the dark (Table 2). The iPSCs were counterstained with DAPI (KeyGEN BioTECH, China) and observed under the confocal microscope (LSM800, Zeiss).

4.3. RNA extraction, cDNA synthesis and real-time RT-PCR

Total RNA was isolated using Trizol (Life Technologies) and cDNA was synthesized using the Reverse-Transcribe Kit (TIANGEN, Beijing, China) according to the manufacturer's instructtions. The expression levels of the pluripotency genes and EB markers were quantified by real-time qRT-PCR with SYBR Premix Ex TaqTM II PCR reagent kit (TaKaRa) on LightCycler 480 II machine (Roche Diagnostics, Mannheim, Germany) and indicated primers (Table 2).

4.4. DNA isolation, PCR and Sanger sequencing

Genomic DNA was extracted following the standard steps. PCR reactions were performed with plasmids specific primers (Table 2) using 2 × ES Taq MasterMix (CWBIO, Beijing, China) for 35cycles (94 °C for 30 s, 58 °C for 30 s and72 °C for 30 s) on PTC-200 Thermal Cycler (BIO-RAD). Mutation in JAG1 gene was detected by Sanger sequencing on a 3130XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

4.5. Karyotyping

The cell line was treated with colchicine (Gibco) for 55 min when it reached 75%–85% confluence. Swelled the collected cells with 0.075 M hypotonic KCl solution followed by fixation with Carnoy's fixative (methanol: Acetic Acid = 3:1). Over 50 metaphase chromosomes were evaluated for the standard G-banded analysis using an automated cytogenetic imaging system at 400 resolutions (Leica, GSL-120).

4.6. In vitro spontaneous differentiation

iPSCs were treated with collagenase IV (Gibco) for about 30 min at 37 °C. The big-sized cell pellets were collected and plated in UltraLow Attachment plate (Corning) with embryoid body (EB) medium (DMEM/F12 (Gibco), 20% Knockout serum replacement (Gibco), 1% L-GlutaMax (Gibco), 1%β-mercaptoethanol (Gibco) and 1% NEAA (Gibco)). The formed EBs were transferred to Matrigel-coated well and cultured for another week.

4.7. Short tandem repeat (STR) analysis

Genomic DNAs were extracted from SDQLCHi037-A and corresponding PBMCs with TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). PCR amplification of 20 distinct STRs for the DNA typing were performed using the GoldenEye 20A kit (Peoplespot, Beijing, China) on a 3130XL Analyzer and analyzed by GeneMapper ID version3.2 (Applied Biosystems).

4.8. Mycoplasma detection

Absence of mycoplasma contamination was confirmed by DNA-specific fluorescent dye Hoechst 33258 (KeyGEN BioTECH, China).

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102162.

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