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Characterization of an iPSC line NCHi006-A from a patient with hypoplastic left heart syndrome (HLHS)

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Abstract

Hypoplastic left heart syndrome (HLHS) is a severe congenital heart defect characterized by underdeveloped structures on the left side of the heart, including hypoplasia of the left ventricle and stenosis or atresia of the aortic and mitral valves. Here, we generated an iPSC line from the peripheral blood mononuclear cells of a male patient with HLHS through Sendai virus-mediated transfection of 4 Yamanaka factors. This iPSC line exhibited normal morphology, expressed pluripotency markers, had a normal karyotype, and could differentiate into cells of three germ layers. This iPSC line can be used for studying cellular and developmental etiologies of HLHS.

1. Resource Table

Unique stem cell line identifier	NCHi006-A
Alternative name(s) of stem cell line	NCH023 (NCHi006-A)
Institution	Center for Cardiovascular Research, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, USA Mingtao Zhao, PhD
Contact information of distributor	Mingtao.Zhao@nationwidechildrens.org
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 5 months Ethnicity: Caucasian Sex: male

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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.2022.102892.

Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Method of reprogramming	A Sendai virus vector including human KLF4, OCT3/4, SOX2, and c-MYC
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR and agarose gel electrophoresis: negative for KOS transgene (Supplementary Fig. 1B)
Associated disease	Hypoplastic Left Heart Syndrome (HLHS)
Gene/locus	N/A
Date archived/stock date	01/01/2022
Cell line repository/bank	NCHi006-A (NCH023) is deposited in the iPSC repository of pediatric cardiovascular disease in the Center for Cardiovascular Research at the Abigail Wexner Research Institute at Nationwide Children's Hospital in Columbus, OH, USA. https://hpscreg.eu/cell-line/NCHi006-A
Ethical approval	Generation of this iPSC line was under an approved Institutional Review Board (IRB) protocol STUDY00001788 "iPSC Repository of Pediatric Cardiovascular Disease" at Nationwide Children's Hospital.

2. Resource utility

Derived from a patient with hypoplastic left heart syndrome (HLHS), this iPSC line can be differentiated into cardiac cell lineages for modeling congenital heart defects. It may serve as a useful in vitro biological system to study underlying mechanisms of HLHS, screen for candidate therapeutics, and increase our understanding of human cardiac development.

3. Resource details

Hypoplastic left heart syndrome is a severe congenital heart defect where the left ventricle of the heart is underdeveloped, causing aberrant hemodynamics (Grossfeld et al., 2019). The cause of HLHS is still poorly understood due to the lack of experimental models. Here, we established and characterized an iPSC line derived from a male infant with HLHS to provide a biological system that retains the genetic information of the proband and can be utilized to model stages of human development through differentiation into the three germ layers. We envision this iPSC line to be used as a patient-specific biological model to study human cardiac development, especially to interrogate the mechanisms that govern the pathogenesis of HLHS (Hall et al., 2022; Lin et al., 2021).

To generate iPSC line NCHi006-A, blood was drawn from a patient clinically diagnosed with HLHS and mitral/aortic stenosis, with no other observed heart defects (see Table 1). The peripheral blood mononuclear cells (PBMCs) were isolated and transfected with 4 Yamanaka factors to produce an iPSC line that exhibited normal morphology and colony formation (Fig. 1A). The majority of cells displayed pluripotency markers TRA-1–60, SOX2, NANOG, and OCT3/4, as detected by immunofluorescence staining (Fig. 1B & D). Genetically, the iPSCs displayed a normal male karyotype (46, XY) as confirmed by a whole-genome array (Fig. 1C), and their identity was confirmed using STR analysis to prove their origin from the patient's PBMCs (**in archive with the journal**). This iPSC line

had the ability to differentiate into cells of all three germ layers, as established by positive immunofluorescence staining of germ layer-specific markers. Ectodermal-like cells showed expression of PAX6 and OTX2, mesodermal-like cells displayed TBX6 and Brachyury, while endodermal-like cells expressed FOXA2 and SOX17 (Fig. 1E). The iPSCs were also tested negative for mycoplasma contamination (Supplementary Fig. 1A).

4. Materials and methods

4.1. Reprogramming

Patient PBMCs were isolated and incubated for one week in StemPro-34 SFM medium (Thermo Fisher Scientific) supplemented with 100 ng/mL SCF (PeproTech), 100 ng/mL FLT3 (Thermo Fisher Scientific), 20 ng/mL IL3 (PeproTech), 20 ng/mL IL6 (Gibco), 20 ng/mL EPO (Thermo Fisher Scientific), and 1× GlutaMAX (Thermo Fisher Scientific). PBMCs were then transfected using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Transfected cells were resuspended in supplemented StemPro-34 SFM medium and transferred into a Matrigel-coated plate for one week. Cells were then switched to complete E8 medium (Thermo Fisher Scientific). After two weeks, emerging iPSC clones were picked, expanded over multiple passages, and stored in liquid N_2.

4.2. iPSC maintenance and passaging

Cells were maintained in complete E8 media at 37 °C with 5% CO_2. Upon reaching 90% confluency, cells were washed with DPBS then dissociated with 0.5 mM EDTA for 5–8 min. EDTA was then removed, and iPSCs were manually dislodged with complete E8 media plus ROCK inhibitor (Y-27632, Selleck Chemicals). To split, the cell suspension was replated at a 1:6–1:10 ratio.

4.3. Immunofluorescent staining

The pluripotency of iPSCs (passages 12–13) was assessed by immunofluorescence staining and manual counting. Cells were fixed with 4% paraformaldehyde solution (Electron Microscopy Sciences) for 15 min, then permeabilized with 0.1% Triton X-100 solution (Sigma) for 20 min at room temperature. Cells were then blocked with 0.2% BSA (Sigma) in DPBS, and incubated with primary antibodies (dilution: 1:200) overnight at 4 °C. The next day, secondary antibodies (dilution: 1:2000) in 0.2% BSA were added at room temperature for 1 h, then counterstained with DAPI (dilution: 1:2000) in DPBS at room temperature for 10 min (see Table 2). Stained coverslips were mounted onto glass slides using SlowFade Gold Antifade (Thermo Fisher Scientific) and imaged with a fluorescence microscope (Keyence).

4.4. Karyotyping

To detect chromosomal abnormalities using whole genome array, 2×10^6 iPSCs (passages 12–13) from were harvested and analyzed using the KaryoStat Assay (Thermo Fisher Scientific).

4.5. Short tandem repeat (STR) analysis

Genomic DNA was extracted from iPSCs (passages 14–15) and PBMCs using the Quick-DNA Miniprep Plus Kit (Zymo Research). The PowerPlex 16 System (Promega) was then utilized to amplify genomic materials according to the manufacturer's instructions. Samples were sent for capillary sequencing using an ABI 3730xl Genetic Analyzer (Thermo Fisher Scientific). GeneMapper 5.0 (Thermo Fisher Scientific) software was used to analyze the sequencing data for allele callings for 16 loci per sample. Only strong allele calling signals were considered for analysis.

4.6. Germ layer differentiation

Pluripotency was confirmed by differentiating iPSCs (passages 11–12) into endoderm and ectoderm cells using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) according to the manufacturer's instructions. Mesoderm differentiation was induced through two-day application of 6 μM CHIR99021 (Selleck Chemicals) in RPMI 1640 media (Thermo Fisher Scientific) with B27 minus insulin supplement (Thermo Fisher Scientific). Samples were fixed and stained with respective germ layer-specific markers.

4.7. Mycoplasma detection

Mycoplasma contamination was checked using the MycoAlert™ Detection Kit (Lonza) on iPSC passage 12 supernatant following the manufacturer's protocol.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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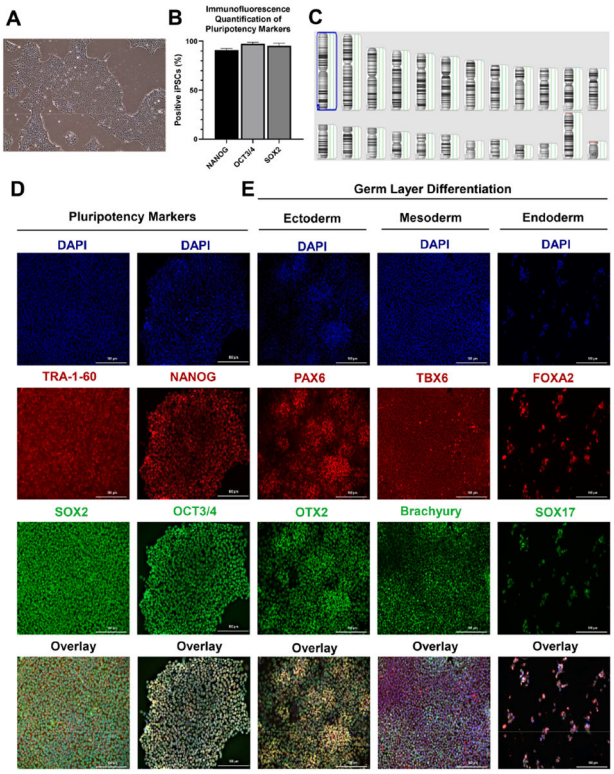

Fig. 1.
Characterization of an iPSC line derived from an HLHS patient.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis: Immunocytochemistry	Expression of TRA-1–60, NANOG, SOX2, OCT3/4	Fig. 1D
	Quantitative analysis: Immunocytochemistry counting	NANOG: 91 ± 2%, OCT3/4: 97 ± 2%, SOX2: 95 ± 2%	Fig. 1B
Genotype	Whole genome array (KaryoStat+ Assay)	Normal karyotype: 46, XY; Resolution 1–2 Mb	Fig. 1C
Identity	Microsatellite PCR (mPCR)	Not performed	N/A
	OR		
	STR analysis	16 loci tested with matching identity	Submitted in archive with journal
Mutation analysis	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Supplementary Figure 1A
Differentiation potential	Trilineage in-vitro differentiation	Positive immunofluorescence staining of three germ layers Ectoderm: PAX6, OTX2, Endoderm: SOX17, FOXA2, Mesoderm: BRACHYURY/TBXT, TBX6,	Fig. 1E
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2

Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Marker	Rabbit anti-NANOG	1:200	Cell Signaling Technology, Cat# 4903P	AB_10559205
Pluripotency Marker	Alexa Fluor 488 Mouse anti-OCT3/4	1:2000	BD Biosciences, Cat# 561628	AB_10895977
Pluripotency Marker	Mouse anti-TRA-1–60	1:200	Thermo Fisher Scientific, Cat# MA1-023X	AB_2536705
Pluripotency Marker	Rabbit anti-SOX2	1:200	Thermo Fisher Scientific, Cat# PA1-094X	AB_2539862
Ectoderm Marker	Goat anti-OTX2	1:200	R&D Systems, Cat# AF1979	AB_2157172
Ectoderm Marker	Rabbit anti-PAX6	1:200	Thermo Fisher Scientific, Cat# 42-6600	AB_2533534
Mesoderm Marker	Goat anti-Brachyury	1:200	R&D Systems, Cat# AF2085	AB_2200235
Mesoderm Marker	Rabbit anti-TBX6	1:200	Thermo Fisher Scientific, Cat# PA5-35102	AB_2552412
Endoderm Marker	Goat anti-SOX17	1:200	R&D Systems, Cat# AF1924	AB_355060
Endoderm Marker	Mouse anti-FOXA2	1:200	Abnova, Cat# H00003170-M10	AB_534871
Secondary Antibody	Goat anti-Mouse IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fisher Scientific, Cat# A-11032	AB_2534091
Secondary Antibody	Goat anti-Mouse IgG (H + L), Alexa Fluor 488	1:2000	Thermo Fisher Scientific, Cat# A-11001	AB_2534069
Secondary Antibody	Goat anti-Rabbit IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fisher Scientific, Cat# A-11012	AB_2534079
Secondary Antibody	Donkey anti-Mouse IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fisher Scientific, Cat# R37115	AB_2556543
Secondary Antibody	Donkey anti-Rabbit IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fisher Scientific, Cat# R37119	AB_2556547
Secondary Antibody	Donkey anti-Goat IgG (H + L), Alexa Fluor Plus 488	1:2000	Thermo Fisher Scientific, Cat# A32814	AB_2762838
Primers				
	Target	**Band Size**	**Primer Sequence (5′-3′)**	
Housekeeping Gene (RT-PCR)	GAPDH	452 bp	F: ACCACAGTCCATGCCATCAC R: TCCACCACCCTGTTGCTGTA	
Transgene (RT-PCR)	KOS	528 bp	F: ATGCACCGCTACGACGTGAGCGC R: ACCTTGACAATCCTGATGTGG	