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## Generation of an induced pluripotent stem cell line NCHi003-A from a 11-year-old male with pulmonary atresia with intact ventricular septum (PA-IVS)

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### Abstract

Pulmonary atresia with intact ventricular septum (PA-IVS) is a rare congenital heart defect defined by membranous or muscular atresia of the right ventricular outflow tract where patients display varying degrees of hypoplasia of the right ventricle. This condition results in cyanosis due to an inability of blood to flow from the right ventricle to the pulmonary arteries, thus requiring immediate surgical intervention after birth. An iPSC line was generated from peripheral blood mononuclear cells of a 11-year-old male patient diagnosed with PA-IVS through Sendai virus-mediated reprogramming. This disease-specific iPSC line was characterized by immunocytochemistry, STR analysis, karyotype analysis, and mycoplasma testing.

### 1. Resource utility

An iPSC line (Resource Table) was generated from a patient with pulmonary atresia with intact ventricular septum using Sendai virus-mediated epigenetic reprogramming. Differentiated cardiac cells from this PA-IVS-specific iPSC line could serve as clinically relevant cell sources for modelling congenital heart disease and novel drug discovery (Lin et al., 2021).

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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.2022.102893>.

## 2. Resource details

PA-IVS is a rare congenital heart defect driven primarily by varying degrees of hypoplasia of the right ventricle and membranous or muscular atresia of the right ventricular outflow tract that results in cyanosis (Gorla and Singh, 2022; Rao, 2021; Wright et al., 2019). PA-IVS carries a significant risk of early morbidity and mortality that warrants immediate intervention after birth (Rao, 2021; Wright et al., 2019). The cause of PA-IVS is poorly understood and patient cell-based experimental models are not available (Hall et al., 2022). Here, an iPSC line was generated from the peripheral blood mononuclear cells (PBMCs) of a male patient with PA-IVS and verified to model stages of human development through differentiation into cells from the three germ layers.

An iPSC line was generated from the PMBCs of a patient diagnosed with PA-IVS through Sendai virus vector reprogramming (see Table 1). These PA-IVS iPSCs displayed typical stem cell morphology and colony formation (Fig. 1A). Immunofluorescence staining indicated the expression of pluripotency markers OCT3/4, NANOG, SOX2, TRA-1-60, in the majority of iPSCs (Fig. 1B and D). These iPSCs displayed a normal male karyotype (46, XY) that was confirmed by a whole genome array (Fig. 1C). *In vitro* germ layer differentiation showed positive staining for layer-specific markers, with endoderm-derived cells expressing FOXA2 and SOX17, mesoderm-derived cells expressing TBX6 and Brachyury, and ectoderm-derived cells expressing PAX6 and OTX2 (Fig. 1E). This PA-IVS iPSC line was tested negative for mycoplasma (Supplementary Fig. 1). In addition, short tandem repeat (STR) analysis confirmed that this iPSC line was originated from the PA-IVS patient (in archive with the journal).

## 3. Materials and methods

### 3.1. Reprogramming and iPSC culture

Patient PBMCs were isolated and incubated for one week in StemPro-34 SFM medium (Thermo Fischer Scientific) that was supplemented with 100 ng/mL SCF (PeproTech), 100 ng/mL FLT3 (Thermo Fischer Scientific), 20 ng/mL IL3 (PeproTech), 20 ng/mL IL6 (Gibco), 20 ng/mL EPO (Thermo Fischer Scientific), and 1X GlutaMAX (Thermo Fischer Scientific). PBMCs were infected using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fischer Scientific) according to the manufacturer's protocol. Transduced cells were resuspended in the supplemented StemPro-34 SFM medium and transferred into a Matrigel-coated plate for one week. Medium was then changed to Essential 8 (E8) medium (Thermo Fischer Scientific). After two weeks, emerging iPSC colonies were picked, expanded over multiple passages, and cryopreserved in a liquid nitrogen tank.

iPSCs were maintained in a CO<sub>2</sub> incubator at 37 °C and 5 % CO<sub>2</sub> in E8 medium. Media was refreshed daily. iPSCs were dissociated using 0.5 mM EDTA and passaged onto a Matrigel-coated plate with E8 medium supplemented with ROCK inhibitor (Y-27632, Selleck Chemicals).

### 3.2. Immunofluorescent staining

iPSCs (p43) were cultured on Matrigel-coated coverslips in a 24-well plate for 2 days. Cells were fixed with 4 % paraformaldehyde (PFA, Electron Microscopy Sciences) for 15 min. After permeabilization with 0.1 % Triton-X-100 (Sigma) solution for 20 min, cells were blocked with 1X DPBS/0.2 % Bovine Serum Albumin (BSA, Sigma) for 1 h at room temperature. Cells were incubated with primary antibodies diluted in 0.2 % BSA solution overnight at 4 °C (see Table 2). After washing 3 times, cells were then incubated with secondary antibodies for 1 h in the dark. Cells were washed 3 times using 1X DPBS, and counterstained with DAPI solution for 10 min. Coverslips were mounted onto slides with SlowFade Gold Antifade (Thermo Fischer Scientific) and imaged with a fluorescence microscope (Keyence). All steps were performed at room temperature unless otherwise described.

### 3.3. Short tandem repeat (STR) analysis

Genomic DNA of iPSCs (p52) and patient's PBMCs was extracted using Quick-DNA Miniprep Plus Kit (Zymo Research). STRs were amplified using the PowerPlex16 (Promega) system and resolved on a ABI 3730xl Genetic Analyzer (Thermo Fischer Scientific). GeneMapper 5.0 (Thermo Fischer) software was used to call the genotype at each locus and compare between peripheral blood and iPSC samples.

### 3.4. In vitro differentiation potential

The differentiation potential of iPSCs (p46) was verified through cell differentiation into the three germ layers by measuring the expression of endoderm (OTX2, PAX6), mesoderm (Brachyury, TBX6), and ectoderm (SOX17, FOXA2) markers. Cells of endoderm and ectoderm lineages were generated using a Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems). Cells of mesoderm layers were induced with 6  $\mu$ M CHIR99021 (Selleck Chemicals) in RPMI 1640 media (Thermo Fischer Scientific) with B27 Supplement minus insulin (Thermo Fischer Scientific) for 2 days. Samples were fixed and stained for their respective markers.

### 3.5. Karyotype analysis

Human iPSCs (p61) were harvested and sent for KaryoStat+ Assay Service provided by Thermo Fisher Scientific.

### 3.6. Mycoplasma detection

Supernatant of dense iPSC cultures (p45) was tested using the MycoAlert™ Mycoplasma Detection Kit (Lonza Biosciences).

## Supplementary Material

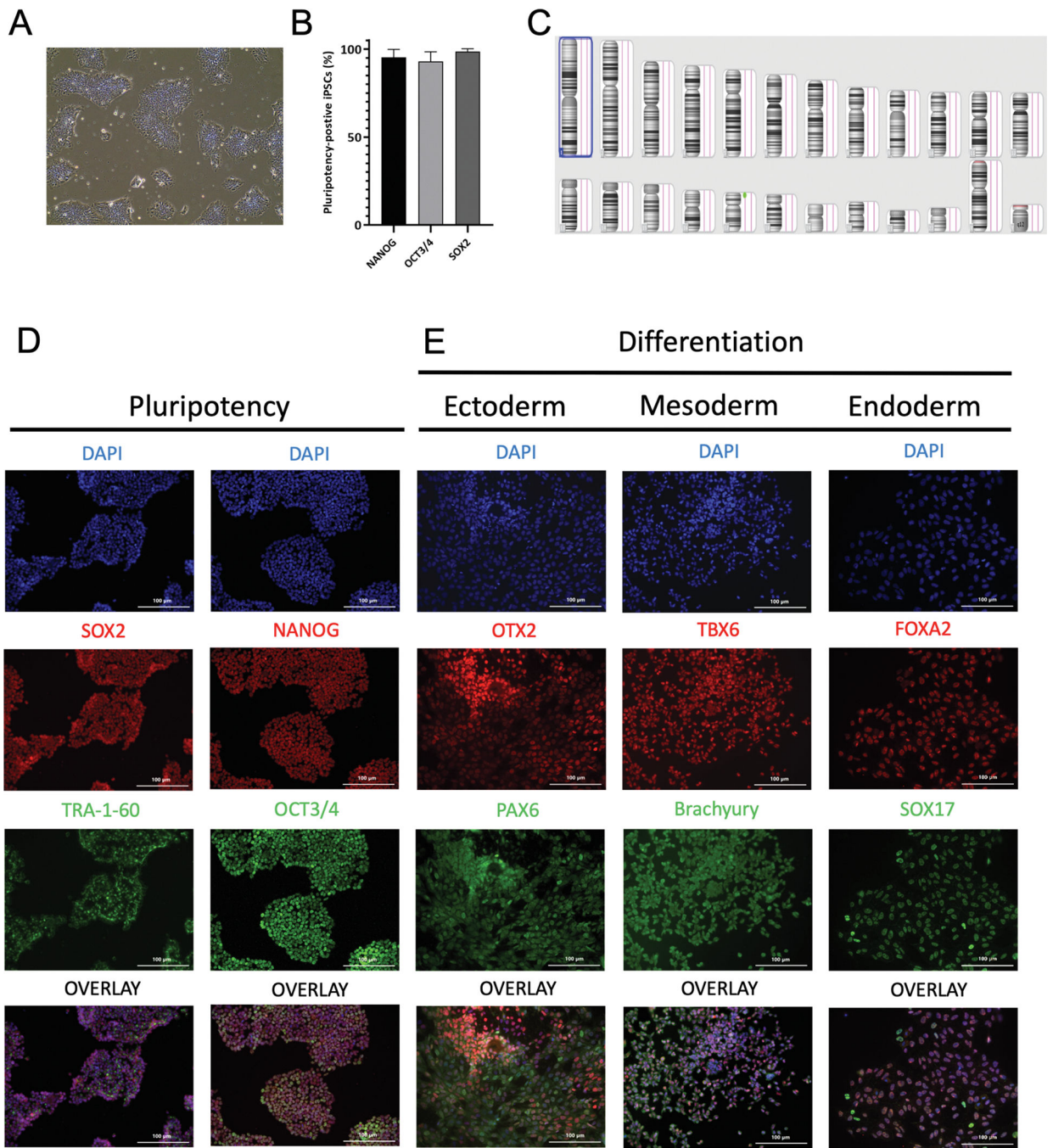
Refer to Web version on PubMed Central for supplementary material.

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**Fig. 1.**  
 Characterization of an iPSC line derived from a PA-IVS patient.

**Table 1**

Characterization and validation.

Classification	Test	Result	Data
<b>Morphology</b>	Photography, bright field	Normal	Fig. 1A
<b>Phenotype</b>	Qualitative analysis	Positive expression of OCT3/4, Nanog, Sox2, TRA-1-60 markers	Fig. 1D
	Quantitative analysis	OCT3/4: 93 % $\pm$ 5 %	Fig. 1B
		NANOG: 95 % $\pm$ 5 % SOX2: 98 % $\pm$ 2 %	
<b>Genotype</b>	Whole genome array (KaryoStat Assay)	Normal karyotype: 46, XY Resolution 1-2 Mb	Fig. 1C
<b>Identity</b>	Microsatellite PCR (mPCR) OR STR analysis	mPCR not performed	N/A
<b>Mutation analysis (IF APPLICABLE)</b>	Sequencing	16 loci tested with matching identity	Submitted in archive with journal
	Southern blot OR WGS	N/A	N/A
<b>Microbiology and virology</b>	Mycoplasma	N/A	N/A
	Trilineage in vitro differentiation	Negative result	Supplementary Fig. 1
<b>Differentiation potential</b>		Positive staining for three germ layers: Endoderm: SOX17, FOXA2 Mesoderm: Brachyury, TBX6 Ectoderm: PAX6, OTX2	Fig. 1E
	HIV 1 + 2, Hepatitis B, Hepatitis C	N/A	N/A
	Blood group genotyping	N/A	N/A
<b>Donor screening</b>	HLA tissue typing	N/A	N/A
<b>Genotype additional info</b>		N/A	N/A
		N/A	N/A

**Table 2**

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
Antibody	Dilution	Company Cat #	RRID	
Pluripotency Markers	Alexa Fluor 488 Mouse anti-OCT3/4	1:2000	BD Biosciences Cat No: 561.628	AB_10895977
Pluripotency Markers	Rabbit anti-NANOG	1:200	Cell Signaling Technology Cat No: 4903P	AB_10611718
Pluripotency Markers	Mouse anti-TRA-1-60	1:200	Thermo Fischer Scientific Cat No: MA1-023X	AB_2536705
Pluripotency Markers	Rabbit anti-SOX2	1:200	Thermo Fischer Scientific Cat No: PA1-094X	AB_2539863
Endoderm Marker	Goat anti-SOX17	1:200	R&D Systems Cat No: AF1924	AB_355060
Endoderm Marker	Mouse anti-FOXA2	1:200	Abnova Cat No: H00003170-M10	AB_534871
Mesoderm Marker	Goat anti-Brachyury	1:200	R&D Systems Cat No: AF2085	AB_2200235
Mesoderm Marker	Rabbit anti-TBX6	1:200	Thermo Fischer Scientific Cat No: PA5-35102	AB_2552412
Ectoderm Marker	Goat anti-OTX2	1:200	R&D Systems Cat No: AF1979	AB_2157172
Ectoderm Marker	Rabbit anti-PAX6	1:200	Thermo Fischer Scientific Cat No: 42-6600	AB_2533534
Secondary Antibody	Goat anti-Mouse IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fischer Scientific Cat# A-11032	AB_2534091
Secondary Antibody	Goat anti-Mouse IgG (H + L), Alexa Fluor 488	1:2000	Thermo Fischer Scientific Cat# A-11001	AB_2534069
Secondary Antibody	Goat anti-Rabbit IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fischer Scientific Cat# A-11012	AB_2534079
Secondary Antibody	Donkey anti-Mouse IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fischer Scientific Cat# R37115	AB_2556543
Secondary Antibody	Donkey anti-Rabbit IgG (H + L), Alexa Fluor 594	1:2000	Thermo Fischer Scientific Cat# R37119	AB_2556547
Secondary Antibody	Donkey anti-Goat IgG (H + L), Alexa Fluor Plus 488	1:2000	Thermo Fischer Scientific Cat# A32814	AB_2762838

**Resource Table**

Unique stem cell line identifier	NCHi003-A
Alternative name(s) of stem cell line	NCH005 (NCHi003-A)
Institution	Center for Cardiovascular Research, Abigail Wexner Research Institute, Nationwide Children’s Hospital, Columbus, OH, 43,215
Contact information of distributor	Dr. Ming-Tao Zhao mingtao.zhao@nationwidechildrens.org
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 11 years Sex: male Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Sendai virus reprogramming 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)	N/A
Associated disease	Pulmonary Atresia with Intact Ventricular Septum
Gene/locus	N/A
Date archived/stock date	June 17, 2022
Cell line repository/bank	NCHi003-A (NCH005) 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, Ohio, USA. <a href="https://hpscereg.eu/cell-line/NCHi003-A">https://hpscereg.eu/cell-line/NCHi003-A</a>
Ethical approval	This study was approved by the Internal Review Board (IRB) - STUDY00001788 at Nationwide Children’s Hospital.

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