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Establishment of NCHi009-A, an iPSC line from a patient with hypoplastic left heart syndrome (HLHS) carrying a heterozygous NOTCH1 mutation

Subhodip Adhicary[a,b], **Shiqiao Ye**[a,b], **Hui Lin**[a,b], **Karen Texter**[b,c], **Vidu Garg**[a,b,c], **Ming-Tao Zhao**[a,b,c,*]

[a]Center for Cardiovascular Research, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, USA

[b]The Heart Center, Nationwide Children's Hospital, Columbus, OH, USA

[c]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, OH, USA

Abstract

Hypoplastic left heart syndrome (HLHS) is a congenital heart malformation clinically characterized by an underdeveloped left ventricle, mitral or aortic valve stenosis or atresia, and narrowed ascending aorta. Although genetic etiology of HLHS is heterogenous, recurrent *NOTCH1* variants have been associated with this defect. We report generation of an iPSC line derived from a female with HLHS with a heterozygous missense *NOTCH1* (c.2058G > A; p.Gly661Ser) mutation within the conserved EGF-like repeat 17. This iPSC line exhibited typical cellular morphology, normal karyotype, high expression of pluripotent markers, and trilineage differentiation potential; and can be leveraged to dissect the complex NOTCH1-mediated HLHS disease mechanism.

Unique stem cell line identifier	NCHi009-A
Alternative name(s) of stem cell line	NCH78
Institution	Center for Cardiovascular Research, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH
Contact information of distributor	Mingtao Zhao, PhD
Type of cell line	iPSC
Origin	Human

*Corresponding author at: Center for Cardiovascular Research, Abigail Wexner Research Institute, Nationwide Children's Hospital, Columbus, OH, USA. Mingtao.Zhao@nationwidechildrens.org (M.-T. Zhao).

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.2022.103013.

Additional origin info required for human ESC or iPSC	Age: 20 years Sex: Female Ethnicity if known: Caucasian
Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Sendai virus vector expressing human KLF4, OCT3/4, SOX2, and c-MYC
Genetic Modification	Yes
Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR for *Kos* transgene (Supplementary Fig. 1B)
Associated disease	Hypoplastic left heart syndrome (HLHS)
Gene/locus	*NOTCH1*: c.2058G > A (p.Gly661Ser) / chr9:136515323
Date archived/stock date	31st October 2022
Cell line repository/bank	NCHi009-A (NCH78) 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/NCHi009-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.

1. Resource utility

NCHi009-A is derived from a 20 year old female with hypoplastic left heart syndrome (HLHS) carrying a heterozygous c.2058G > A (p. Gly661Ser) *NOTCH1* variant (Resource Table). This iPSC line provides an unlimited source for differentiated HLHS patient-specific cardiomyocytes to study NOTCH1-mediated disease phenotype, and for therapeutic screening (See Table 1).

2. Resource details

HLHS is a congenital heart defect (CHD) characterized by varying degrees of underdevelopment of the structures in the left-ventricular complex, accompanied by anomalous hemodynamic parameters (Barron et al., 2009). Most cases of HLHS are sporadic, although recurrence of de novo mutations in *NOTCH1* have been identified (Iascone et al., 2012; Lin et al., 2021; McBride et al., 2008). However, the molecular mechanisms underlying disease pathogenesis remain elusive. Moreover, the condition is fatal without surgical intervention (Barron et al., 2009), and pharmacological therapy is lacking. CHD patient derived iPSCs with retained genomic information of the proband provide a unique tool to advance our understanding of cardiac development and CHD etiology. NOTCH1 is a single-pass transmembrane receptor that drives the evolutionarily conserved Notch signaling pathway. The extracellular domain of NOTCH1 consists of Epidermal Growth Factor (EGF)-like repeats that primarily aid in ligand interaction dynamics and are sites of posttranslational modifications (Bray, 2016).

Here we generated iPSC line NCHi009-A from peripheral blood mononuclear cells (PBMCs) from a patient diagnosed with HLHS, with a heterozygous *NOTCH1*: c.2058G

> A (p.Gly661Ser) mutation within EGF-like repeat 17. Ectopic expression of this *NOTCH1* variant shows reduced NOTCH1 induction via the ligand JAGGED1 (McBride et al., 2008). Isolated PBMCs were reprogrammed using Sendai viral vector including four Yamanaka factors - Oct3/4, Sox2, Klf4, and L-Myc. NCHi009-A exhibited typical iPSC morphology (Fig. 1A). Presence of heterozygous *NOTCH1*: c.2058G > A (p.Gly661Ser) mutation was confirmed by Sanger sequencing (Fig. 1B). Karyotype integrity was assessed using whole-genome array and the line displayed a normal female karyotype (46, XX) with no chromosomal aberrations (Fig. 1C). Short tandem repeats (STR) analysis of iPSCs and PBMCs confirmed identical genetic origin (data archived). NCHi009-A also tested negative for Mycoplasma (Supplementary Fig. 1A) and was free of Sendai viral genome at passage 14, as assessed via RT-PCR for the viral Kos transgene (Supplementary Fig. 1B). Most cells expressed pluripotency markers SOX2, NANOG, OCT3/4, TRA-1-60 as detected by immunofluorescence (Fig. 1E) and quantified in (Fig. 1D). Furthermore, the iPSC line differentiated into three germ-layers as evidenced by immunofluorescence for lineage specific markers. Ectoderm differentiation was detected using expression of OTX2 and PAX6; mesoderm cells expressed Brachyury and TBX6, and endoderm derived cells were detected using expression of SOX17 and FOXA2 (Fig. 1F).

3. Materials and methods

3.1. Reprogramming and iPSC culture

Proband PBMCs were isolated in separation tubes (BD Biosciences) and incubated in StemPro-34 SFM medium (ThermoFisher Scientific) supplemented with 20 ng/mL IL3 (PeproTech), 20 ng/mL IL6 (Gibco), 20 ng/mL EPO (ThermoFisher Scientific), 100 ng/mL SCF (PeproTech), 100 ng/mL FLT3 (ThermoFisher Scientific) and 1X GlutaMAX (ThermoFisher Scientific), for 7 days. PBMCs were then transduced using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) following manufacturer's instructions. Transduced cells were resuspended in supplemented StemPro-34 SFM medium and transferred to a Matrigel-coated plate for 7 days, after which the medium was replaced with Essential 8 (E8) medium (ThermoFisher Scientific). Emerging iPSC colonies were picked and expanded over passages. iPSCs were maintained in E8 medium under 5 % CO2, at 37 °C. Upon reaching ~ 90 % confluency, cells were dissociated with 0.5 mM EDTA, split at a 1:6–1:8 ratio, and passaged in E8 medium supplemented with ROCK inhibitor (Y-27632, Selleck Chemicals). iPSCs were cryopreserved in liquid nitrogen.

3.2. Karyotyping

Approximately 2×10^6 iPSCs (passage 14) were harvested and karyotype analysed using whole-genome array KaryoStat™ Assay (ThermoFisher Scientific).

3.3. Short tandem repeat (STR) analysis

Genomic DNA (gDNA) was extracted from iPSCs (passages 14–20) and PBMCs using the Quick-DNA Miniprep Plus Kit (Zymo Research). Genetic markers with distinguishable alleles were analysed to determine identity using the PowerPlex16 System (Promega). gDNA was amplified using primer-mix for 16 polymorphic markers, and capillary

sequenced on ABI 3730xl Genetic Analyzer (ThermoFisher Scientific). Fluorescent signals were analysed using the GeneMapper 6.0 (Applied Biosystems) program.

3.4. DNA sequencing

gDNA extracted from iPSCs and PBMCs was PCR amplified using primers flanking the NOTCH1 mutation (Table 2) using a hot-start, highfidelity DNA polymerase (Takara). Amplicons were electrophoresed on a 1.5 % agarose gel to validate product specificity. PCR product was purified using the DNA Clean & Concentrator® kit (Zymo Research) and Sanger sequenced.

3.5. RT-PCR

Total RNA was extracted using the Direct-zol RNA Miniprep Kits (Zymo Research) and first-strand cDNA synthesized using the iScript™ cDNA Synthesis Kit (BioRad). cDNA was PCR amplified for detection of Sendai viral *Kos* transgene, and human *GAPDH* was used as a positive control (See Table 2 for primers). PCR amplification was performed on a VeritiPro™ thermal cycler (Applied Biosystems). Cycling conditions followed 30 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 15 s and extension at 68 °C for 1 min. Amplicons were electrophoresed in a 1.5 % agarose gel.

3.6. Immunofluorescent staining

iPSCs (passage 14–20) were cultured on Matrigel-coated coverslips in a 24-well plate. Cells were fixed with 4 % paraformaldehyde (Electron Microscopy Sciences) for 15 min, permeabilized with 0.1 % Triton-X-100 (Sigma) solution for 20 min and blocked with 0.3 % BSA (Sigma) solution for 1 h at room temperature. Cells were then incubated with primary antibodies diluted in 0.3 % BSA solution overnight at 4 °C (Table 2), and appropriate secondary antibodies for 1 h, and counterstained with DAPI for 10 min. Coverslips were mounted onto slides with SlowFade Gold Antifade (ThermoFisher Scientific) and imaged with an epifluorescence microscope (Keyence).

3.7. Germ layer differentiation

iPSCs (passages 14–20) were differentiated into endoderm and ectoderm lineages using the Human Pluripotent Stem Cell Functional Identification Kit (R&D Systems) according to manufacturer's instructions. Mesoderm differentiation was induced via 2-day application of 6 μM CHIR99021 (Selleck Chemicals) in RPMI 1640 (ThermoFisher Scientific) with B27 minus insulin supplement (ThermoFisher Scientific). Samples were immunostained with respective germ layer-specific markers (Table 2).

3.8. Mycoplasma detection

Mycoplasma contamination was checked using the MycoAlert™ Detection Kit (Lonza) on iPSC passage 14 spent media following manufacturer's instructions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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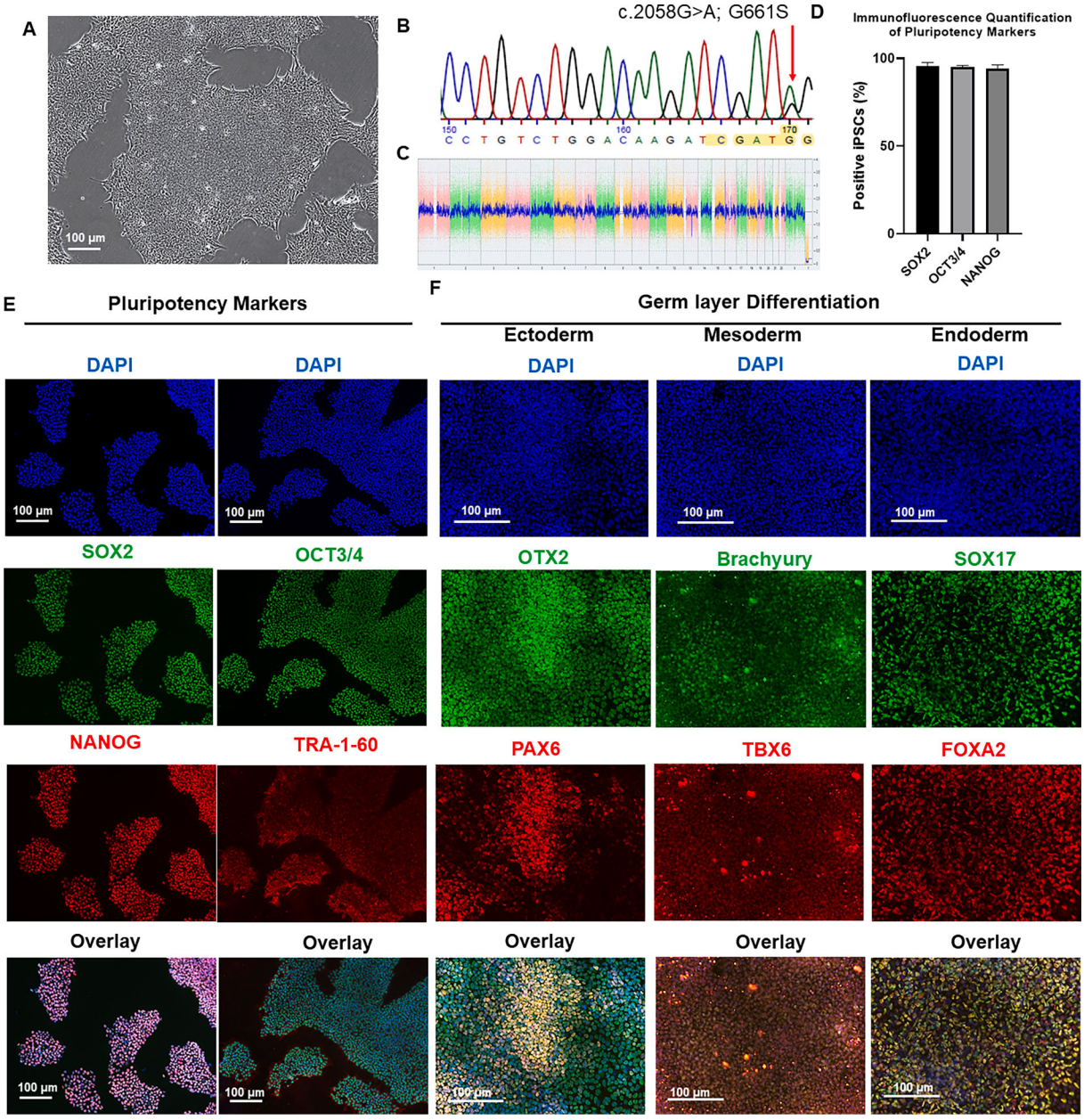

Fig. 1.

Characterization of NCHi009-A derived from an HLHS patient with NOTCH1 mutation.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography, bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis Immunocytochemistry	Expression of SOX2, OCT3/4, NANOG, TRA-1–60	Fig. 1E
	Quantitative analysis Immunocytochemistry Count	SOX2: 95 % ± 2 % OCT3/4: 95 % ± 1 % NANOG: 94 % ± 2 %	Fig. 1D
Genotype	Whole genome array (KaryoStat + Assay)	Normal karyotype: 46, XX; Resolution 1–2 Mb	Fig. 1C
Identity	Microsatellite PCR (mPCR) OR	Not performed	
	STR analysis	16 loci tested with matching identity	Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous	Fig. 1B
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Luminescence Negative	Supplementary Fig. 1A
Differentiation potential	Trilineage in-vitro differentiation	Positive immunofluorescence staining of three germ layers	Fig. 1F
		Ectoderm: PAX6, OTX2	
		Mesoderm: BRACHYURY/TBXT, TBX6	
		Endoderm: SOX17, FOXA2	
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

Table 2

Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Marker	Rabbit anti-NANOG	1:500	Cell Signaling Technology,Cat#4903P	AB_10559205
	Rabbit anti-SOX2	1:500	Thermo Fisher Scientific, Cat# PA1-094X	AB_2539862
	Alexa Fluor 488 Mouse anti-OCT3/4	1:500	BD Biosciences, Cat# 561628	AB_10895977
	Mouse anti-TRA-1-60	1:500	Thermo Fisher Scientific, Cat# MA1-023X	AB_2536705
Ectoderm Marker	Goat anti-OTX2	1:500	R&D Systems, Cat# AF1979	AB_2157172
	Rabbit anti-PAX6	1:500	Thermo Fisher Scientific, Cat# 42-6600	AB_2533534
Mesoderm Marker	Goat anti-Brachyury	1:500	R&D Systems, Cat# AF2085	AB_2200235
	Rabbit anti-TBX6	1:500	Thermo Fisher Scientific, Cat# PA5-35102	AB_2552412
Endoderm Marker	Goat anti-SOX17 Mouse anti-FOXA2	1:500 1:500	R&D Systems, Cat# AF1924 Abnova, Cat# H00003170-M10	AB_355060 AB_534871
Secondary antibodies	Goat anti-Mouse IgG (H + L), Alexa Fluor 594	1:1000	e Thermo Fisher Scientific, Cat# A-11032	AB_2534091
	Goat anti-Mouse IgG (H + L), Alexa Fluor 488	1:1000	Thermo Fisher Scientific, Cat# A-11001	AB_2534069
	Goat anti-Rabbit IgG (H + L), Alexa Fluor 594	1:1000	Thermo Fisher Scientific, Cat# A-11012	AB_2534079
	Donkey anti-Mouse IgG (H + L), Alexa Fluor 594	1:1000	Thermo Fisher Scientific, Cat# R37115	AB_2556543
	Donkey anti-Rabbit IgG (H + L), Alexa Fluor 594	1:1000	Thermo Fisher Scientific, Cat# R37119	AB_2556547
	Donkey anti-Goat IgG (H + L), Alexa Fluor Plus 488	1:1000	Thermo Fisher Scientific, Cat# A32814	AB_2762838
	Primers			
	Target	Size of band	Forward/Reverse primer (5′–3′)	
Genotyping	*NOTCH1*: c.2058G>A Heterozygous	401bp	ACAACGCCTACCTCTGCTTC/TGCTGCCAGTTATAGCCCTG	
Transgene (RT-PCR)	*Kos*	528bp	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG	
Housekeeping Gene (RT-PCR)	*GAPDH*	452bp	ACCACAGTCCATGCCATCAC/TCCACCACCCTGTTGCTGTA	