HHS Public Access

Author manuscript

Stem Cell Res. Author manuscript; available in PMC 2023 October 15.

Published in final edited form as:

Stem Cell Res. 2023 June ; 69: 103098. doi:10.1016/j.scr.2023.103098.

Generation of 2 isogenic clones from a patient with Trisomy 21 and a GATA1 mutation

Kaoru Takasaki[1,2], **Sara S. Kumar**[1], **Alyssa Gagne**[1], **Deborah L. French**[1,2], **Stella T. Chou**[1,2]

[1]Children's Hospital of Philadelphia, Philadelphia, PA 19104

[2]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Abstract

Trisomy 21 (T21), or Down Syndrome (DS), is a common chromosomal disorder resulting from a third copy of chromosome 21 (HSA21). Transient myeloproliferative disorder (TMD) is a pre-leukemic condition that occurs only in neonates with DS and is characterized by a mutation in the transcription factor *GATA1* that results in a truncated protein (GATA1s). We generated a pair of isogenic T21 lines derived from a patient with TMD that differ only in GATA1 status. The iPSC lines were characterized for pluripotency, differentiation potential, and genomic stability. These lines are a valuable resource for studying T21 hematopoietic diseases.

Resource Table:

Unique stem cell lines identifier	Cell line 1: CHOPi008-A Cell line 2: CHOPi008-C
Alternative name(s) of stem cell lines	Cell line 1: TMD145.T21.G1S Cell line 2: DS145.T21.G1
Institution	Children's Hospital of Philadelphia
Contact information of distributor	Stella T. Chou, MD chous@chop.edu
Type of cell lines	iPSC
Origin	Human
Additional origin info	Age: 8 days Sex: Male Ethnicity if known: White
Cell Source	Peripheral blood
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic Modification	Yes
Type of Genetic Modification	Spontaneous mutation/chromosomal abnormality (germline Trisomy 21 with and without somatic GATA1 mutation)
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qRT-PCR
Associated disease	Transient myeloproliferative disorder
Gene/locus	CHOPi008-A: GATA1 c.3_4insG (GATA1s) CHOPi008-C: GATA1
Date archived/stock date	August 2022

Cell line repository/bank	https://hpscreg.eu/cell-line/CHOPi008-A https://hpscreg.eu/cell-line/CHOPi008-C
Ethical approval	Children's Hospital of Philadelphia, Committees for the Protection of Human Subjects (IRB), IRB 13-010038

Resource utility

Transient myeloproliferative disorder (TMD) occurs only in neonates with Trisomy 21 (T21) and an acquired GATA1 mutation (*GATA1s*). The patient-derived T21 iPSCs with and without a *GATA1s* mutation can be used as an *in vitro* disease model to isolate the effects of chromosome 21 (HSA21) copy number and GATA1s.

Resource Details

TMD is a pre-leukemic condition that affects ~10% of neonates with Down syndrome (DS). TMD blasts contain somatic mutations in *GATA1*, an essential transcription factor for erythroid and megakaryocyte development, resulting in the truncated protein GATA1s. In contrast, non-T21 patients with germline *GATA1* mutations who exclusively express GATA1s develop congenital anemia and neutropenia but have no predisposition to leukemia.

Two peripheral blood samples from a patient with T21 who presented with TMD and then at remission were reprogrammed using Sendai virus expressing the Yamanaka factors (*OCT4, SOX2, KLF4, c-MYC*). Colonies were manually isolated, then screened for characteristic pluripotent stem cell morphology (Figure 1A) and expression of intracellular (Figure 1B) and surface (Figure 1C) pluripotency markers. Sanger sequencing of *GATA1* showed that CHOPi008-A, derived from the TMD blasts, harbors a 1bp insertion (c.3_4insG) resulting in a premature stop codon, and CHOPi008-C, derived from the remission sample, contains wild-type GATA1 (wtGATA1). (Figure 1D). G-band karyotyping confirmed a male T21 karyotype, 47,XY+21, for both lines (Figure 1E). Short tandem repeat (STR) analysis at 24 loci identified a 100% allele match between wtGATA1 and GATA1s clones (Supp. File 1). Sendai virus clearance (Figure 1F) and negative Mycoplasma testing were confirmed (Supp. Fig. 1). Differentiation by embryoid body formation demonstrated both lines can differentiate into the three germ layers *in vitro* (Figure 1G).

These results demonstrate the successful generation of two isogenic iPSC lines from a patient with T21 differing only by *GATA1* mutation status. The iPSC lines provide a valuable *in vitro* model for understanding the genetic basis for TMD and the hematopoietic abnormalities of DS.

Materials and Methods

Sample collection and processing

Peripheral mononuclear cells (PBMCs) from a patient with T21 (Sample 1: TMD, Sample 2: remission) were isolated as previously described (Maguire et al, 2016).

Generation and culture of iPSCs

PBMCs were reprogrammed as previously described (Yang et al, 2012); samples were expanded in QBSF-60 with SCF (100 ng/mL), IL-3 (10 ng/mL), TPO (50 ng/mL), Flt3 (50 ng/mL), dexamethasone (1.5 uM), ascorbic acid (50 ng/mL), glutamine (1%), and penicillin/streptomycin (1%). Samples 1 and 2 were expanded for 2 and 4 days, respectively. Cells were transduced with Sendai virus expressing Oct3/4, Sox2, Klf4, and cMyc (ThermoFisher) according to manufacturer protocol, then plated on irradiated feeder mouse embryonic fibroblasts (MEFs). Cells were maintained at 37°C, 5% CO_2, and 5% O_2 in DMEM/F12 (80%), knockout serum replacement (20%), glutamine (1%), non-essential amino acids (1%), penicillin/streptomycin (1%), beta-mercaptoethanol (0.1 mM), and bFGF (10 ng/mL); media was replenished every 2-3 days for 3 weeks until colonies were manually isolated and expanded. iPSCs were isolated with TrypLE for analyses.

Flow cytometry

iPSCs (passage ≥15) or differentiated cells were incubated at room temperature x 20m with fluorescently-conjugated antibodies in FACS buffer (Table 1). Cells were fixed with 1% paraformaldehyde and analyzed with a CytoFLEX Flow Cytometer (Beckman Coulter) and FlowJo Software (BD Biosciences).

Immunohistochemistry

Immunohistochemistry was performed in iPSCs (passage ≥15) as previously described (Maguire et al, 2019), but using Fluoro-Gel II (Electron Microscopy Sciences) to counterstain for DAPI. Cells were imaged with an EVOS XL Core (Invitrogen) at 40x magnification.

Mutation verification

A 387-bp region flanking *GATA1* Exon 2 was PCR-amplified (SimpliAmp Thermal Cycler, Applied Biosystems) on extracted genomic DNA (Purelink Genomic DNA Kit, ThermoFisher) with the following parameters: 95°C x 10m, 35 cycles of 95°C x 30s/58°C x 30s/72°C x 90s, and 4°C hold. Sequencing was performed by Genewiz.

STR analysis and karyotyping

DNA fingerprinting and chromosomal G-band (passage ≥15) analyses were performed by Cell Line Genetics. Twenty cells in metaphase were counted per line; 8 were analyzed with a 500 resolution reported as good.

Sendai clearance

Total RNA was extracted at passage ≥5 (PureLink RNA Micro Kit, Invitrogen) and reverse transcribed to cDNA using random hexamers with Superscript III Reverse Transcriptase (Life Technologies). qRT-PCR (LightCycler-480II, Roche) for the viral backbone and exogenous reprogramming factors (*SEV, KLF4, KOS*, and *cMyc*) and *GAPDH* (control) was performed using TaqMan Fast Advanced Master Mix (Applied Biosystems) and corresponding probes (Table 2).

Trilineage differentiation

Differentiations into mesoderm (Byrska-Bishop et al, 2015) and ectoderm (Telezhkin et al, 2016) were performed as previously described. For endoderm, iPSCs were incubated on MEFs x 72 hours in RPMI (0-48h) or SFD (48-72h) with ascorbic acid (50 μg/mL), monothioglycerol (3 μg/mL), glutamine (2 mM), and ActA (50 ng/mL) with CHIR 99021 (2 μM) (0-24h) or bFGF (5 ng/mL) (24-72h).

Mycoplasma testing

PCR testing for Mycoplasma contamination was performed on gDNA (passage ≥27), with mock (no gDNA), negative (no polymerase), and positive controls using Platinum Green Hot Start PCR Master Mix (ThermoFisher) and the following parameters: 95°C x 10m, 35 cycles of 95°C x 45s/55°C x 30s/72°C x 30s, and 4°C hold. Products were separated on a 1.0% agarose gel and visualized with ethidium bromide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the National Institutes of Health/National Heart, Lung, and Blood Institute (NIH/NHLBI) (R01 HL147879 to S.T.C. and T32 HL0007150-41 to K.T.), Hyundai Scholar Hope Grant (S.T.C), American Society of Hematology Research Training Award for Fellows (ASH RTAF to K.T.), and the Mizuno Fund in Hematology (to K.T.).

References

Byrska-Bishop M, VanDorn D, Campbell AE, Betensky M, Arca PR, Yao Y, Gadue P, Costa FF, Nemiroff RL, Blobel GA, French DL, Hardison RC, Weiss MJ, Chou ST. Pluripotent stem cells reveal erythroid-specific activities of the GATA1 N-terminus. J Clin Invest. 2015 Mar 2;125(3):993–1005. Doi: 10.1172/JCI75714. Epub 2015 Jan 26. [PubMed: 25621499]

Maguire JA, Gagne AL, Jobaliya CD, Gandre-Babbe S, Gadue P, French DL. Generation of human control iPS cell line CHOPWT10 from healthy adult peripheral blood mononuclear cells. Stem Cell Res. 2016 Mar;16(2):338–41. Doi: 10.1016/j.scr.2016.02.017. Epub 2016 Feb 3. [PubMed: 27345999]

Maguire JA, Gagne AL, Gonzalez-Alegre P, Davidson BL, Shakkottai V, Gadue P, French DL. Generation of Spinocerebellar Ataxia Type 2 induced pluripotent stem cell lines, CHOPi002-A and CHOPi003-A, from patients with abnormal CAG repeats in the coding region of the ATXN2 gene. Stem Cell Res. 2019 Jan;34:101361. doi: 10.1016/j.scr.2018.101361. Epub 2018 Dec 10. [PubMed: 30611021]

Telezhkin V, Schnell C, Yarova P, Yung S, Cope E, Hughes A, Thompson BA, Sanders P, Geater C, Hancock JM, Joy S, Badder L, Connor-Robson N, Comella A, Straccia M, Bombau G, Brown JT, Canals JM, Randall AD, Allen ND, Kemp PJ. Forced cell cycle exit and modulation of GABAA, CREB, and GSK3β signaling promote functional maturation of induced pluripotent stem cell-derived neurons. Am J Physiol Cell Physiol. 2016 Apr 1;310(7):C520–41. Doi: 10.1152/ajpcell.00166.2015. Epub 2015 Dec 30. [PubMed: 26718628]

Yang W, Mills JA, Sullivan S, Liu Y, French DL, Gadue P. iPSC Reprogramming from human peripheral blood using sendai virus mediated gene transfer (December 11, 2012), StemBook, ed. The Stem Cell Research Community, StemBook, doi/10.3824/stembook.1.73.1, http://www.stembook.org.

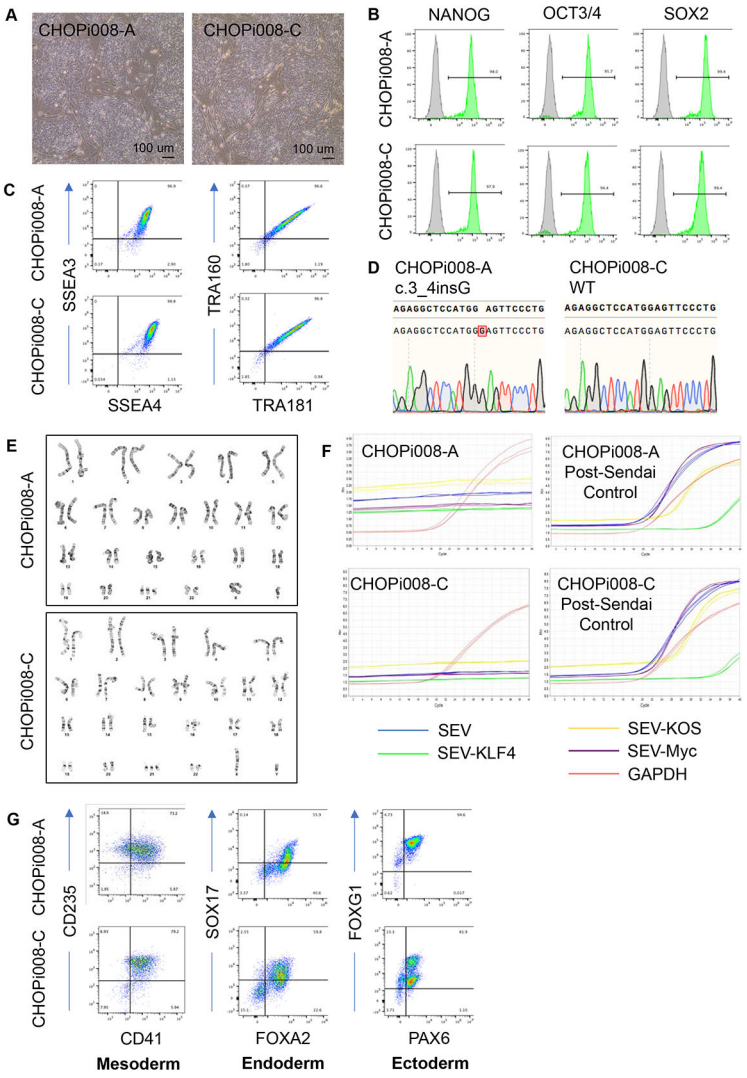

Fig. 1.

Characterization of T21 iPSCs with and without GATA1 mutation.

Table 1:

Characterization and validation

Classification	Test	Result	Data
Morphology	Bright-field photography	Normal	Figure 1A
Phenotype	Qualitative analysis	Positive expression of markers NANOG, OCT4, and SOX2	Figure 1B
	Flow cytometry	CHOPi008-A: SSEA3/4 = 96.9%, Tra-160/181: 96.6% CHOPi008-C: SSEA3/4 = 98.8%, Tra-160/181: 96.8%	Figure 1C
Genotype	Karyotype (G-banding) and resolution	Resolution: 500 CHOPi008-A: 47,XY+21 CHOPi008-C: 47,XY+21	Figure 1D
Identity	Microsatellite PCR (mPCR) OR	N/A	N/A
	STR analysis	24 sites tested and matched on iPSCs and primary patient PBMCs	Supplementary File 1
Mutation analysis	Sequencing	GATA1 on X chromosome, 1bp insertion (c.3_4insG)	Figure 1E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma negative by PCR	Supplementary Figure 1
	Sendai virus	Sendai virus negative by RT-qPCR	Figure 1F
Differentiation potential	Embryoid body formation	Expression of specific markers for ectoderm, mesoderm, and endoderm by flow cytometry	Figure 1G
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	Ectoderm: PAX6, FOXG1 Endoderm: SOX17, FOXA2 Mesoderm: CD41, CD235	Figure 1G
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 Markers	Mouse anti-Oct3/4 (C-10)	1:200	Santa Cruz #sc-5297	RRID:AB_628051
	Rabbit anti-Nanog (D73G4)	1:400	Cell Signaling #4903S	RRID:AB_10559205
	Rabbit anti-Sox2 (D6D9)	1:300	Cell Signaling #3579S	RRID:AB_2195767
	AF488 anti-human SSEA-3	1:50	BioLegend #330306	RRID:AB_1279440
	AF647 anti-human SSEA-4	1:400	BioLegend #330408	RRID:AB_1089200
	AF488 anti-human Tra-1-60	1:100	BioLegend #330614	RRID:AB_2119064
	AF647 anti-human Tra-1-81	1:50	BioLegend #330706	RRID:AB_1089242
Differentiation Markers	PE mouse anti-human Sox17	1:25	BD #561591	RRID:AB_10717121
	Mouse anti-human FoxA2	1:100	Santa Cruz #sc-101060	RRID:AB_1124660
	Rabbit anti-FOXG1	1:300	Abcam #196868	RRID:AB_2892604
	AF647 anti-human PAX6	1:20	BD #562249	RRID:AB_2644844
	PE/Cyanine7 anti-human CD41	1:400	BioLegend #303718	RRID:AB_10899413
	APC Mouse anti-human CD235	1:5000	BD #551336	RRID:AB_398499
Secondary Antibodies	Goat anti-mouse IgG2a-AF647	1:400	Jackson Immunoresearch #115-605-206	RRID:AB_2338917
	Goat anti-rabbit IgG-AF488	1:400	Jackson Immunoresearch #111-545-144	RRID: AB_2338052
	Goat anti-mouse IgG2b-AF488 (flow cytometry)	1:400	Jackson Immunoresearch #115-545-207	RRID:AB_2338856
	Goat anti-Mouse IgG (H+L)-AF488 (immunohistochemistry)	1:400	ThermoFisher #A-11029	RRID:AB_2534088
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai Screening (Taqman qRT-PCR)	SEV	59	Mr04269880_mr	
	SEV-KLF4	67	Mr04421256_mr	
	SEV-KOS	80	Mr04421257_mr	
	SEV-cMYC	89	Mr04269876_mr	
	GAPDH	157	Hs02786624_g1	
Targeted mutation analysis/sequencing	GATA1 Exon 2 screen	387 bp	AGATGCAGGAGGGAAAAGAG / CGGCACATCCATTTGAGAAG	
	GATA1 sequencing		AAGAGGAGCAGGTGAA	
Mycoplasma Detection	16S Ribosomal RNA	518 bp	CGCCTGAGTAGTACGTTCGC / GCGGTGTGTACAAGACCCGA	
	GAPDH (internal control)	150 bp	GTGGACCTGACCTGCCGTCT / GGAGGAGTGGGTGTCGCTGT	