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Stem Cell Res. Author manuscript; available in PMC 2021 August 05.

Published in final edited form as:

Stem Cell Res. 2021 July ; 54: 102432. doi:10.1016/j.scr.2021.102432.

Generation of the iPSC line CUIMCi003-A derived from a patient with severe early onset obesity

Grazia Iannello[a,*], **Achchhe Patel**[b], **Dario Sirabella**[b], **Barbara Corneo**[b], **Vidhu Thaker**[a]

[a]Division of Molecular Genetics, Department of Pediatrics, Columbia University Irving Medical Center, New York, NY, USA

[b]Columbia Stem Cell Initiative, Stem Cell Core, Columbia University Irving Medical Center, NY, USA

Abstract

Aryl hydrocarbon receptor nuclear translocator 2 (*ARNT2*) is a basic helix-loop-helix (bHLH/PAS) transcription factor involved in the development of paraventricular nucleus of the hypothalamus (PVH) through the heterodimerization with Single-minded 1 (*SIM1*) (Michaud et al., 2000). Using a Sendai virus-based approach, the four reprogramming factors OCT3/4, SOX2, KLF4 and C-MYC were delivered into Peripheral Blood Mononuclear Cell (PBMCs) from a 14-year-old girl with early onset obesity carrying a *de novo* variant (p.P130A) in *ARNT2*. The resulting iPSC line CUIMCi003-A had a normal karyotype, showed pluripotency and three germ layer differentiation capacity *in vitro* and was heterozygous for the *de novo ARNT2* variant.

1. Resource table

Unique stem cell line identifier	CUIMCi003-A
Alternative name(s) of stem cell line	*THA20_1*
Institution	*Columbia University Irving Medical Center (CUIMC)*
Contact information of distributor	gi2169@cumc.columbia.edu
Type of cell line	*iPSC*
Origin	*human*
Additional origin info required for human ESC or iPSC	*Age: 14* *Sex: female* *Ethnicity if known: Caucasian*
Cell Source	*Peripheral Blood Mononuclear Cells (PBMCs)*
Clonality	*Clonal*

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*Corresponding author. gi2169@cumc.columbia.edu (G. Iannello).

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.2021.102432.

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Associated disease	*Severe obesity*
Gene/locus	*ARNT2 (p.P130A)/15q25.1*
Date archived/stock date	*May 2021*
Cell line repository/bank	*N/A*
Ethical approval	*Columbia University Irving Medical Center Institutional Research Board under Protocol # AAAS4650. This includes approval from the Human Embryonic and Human Pluripotent Stem Cell Research Committee.*

2. Resource utility

CUIMCi003-A is an iPSC line carrying a heterozygous *de novo* variant (p.P130A) in *ARNT2*, generated from Peripheral Blood Mononuclear Cells (PBMCs) of a patient with severe early onset obesity. This line can be used for disease modelling and drug screening in weight dysregulation studies. By CRISPR/Cas9 correction, CUIMCi003-A can be used to further investigate the genotype-phenotype correlation.

3. Resource details

ARNT2, a transcription factor required for the development of hypothalamus, is highly expressed in the paraventricular hypothalamus (PVH), one of the key regions for weight regulation (Michaud et al., 2000). In this study, a hiPSC line was established from PBMCs of a 14-year-old girl with severe early onset obesity with a heterozygous missense variant in exon 4 of the *ARNT2* (NM_014862.4; c.388C > T) gene. Eight mL of the patient's peripheral blood were collected into BD Vacutainer cell preparation tube (CPT) with sodium citrate and centrifuged (1800 RCF, 30 min, RT) to extract PBMCs. The isolated cells were grown in Expansion Medium (EM): QBSF-60 (cat# 160204101, Quality Biologicals), antibiotics (Primocin, Invivogen, #ant-pm-1; Pen/Strep, Life Technologies, #15140–155; L-Ascorbic Acid, Sigma, # A4544–25G), and growth factors (EPO, #287-TC-500; IL-3, #203-IL-010/CF; IGF-1, #291-G1–200; SCF, #255-SC-010/CF; R&D Systems) and Dexamethasone (Sigma, #D8893–1MG) for few days. After 12 days, 2.5×10^5 cells were collected and reprogrammed into iPSC using a non-integrating Sendai virus approach (CytoTune™-iPS 2.0 Sendai Reprogramming Kit, cat# A16518, ThermoFisher Scientific), with MOI 5:5:3, KOS:c-myc: KLF4 (Yang et al., 2008–2012) following manufacturer's instructions, while the remaining cells were banked. At D12 post-transduction, small colonies with iPSC-like morphology appeared and single clone picking was performed. Each clone was further expanded and the established hiPSC line CUIMCi003-A was selected for further characterization (Fig. 1, Tables 1 and 2). This line exhibited a normal stem cell-like morphology (Fig. 1A, 1000 μm) and G-banded karyotype analysis revealed a normal karyotype (46, XX) (tested at passage 6, Fig. 1B). The absence of Sendai viral transgenes in CUIMCi003-A at passage 17 was confirmed by qRT-PCR: the patient PBMCs after Sendai transduction were used as a positive control, while a previously published iPSC line (Patel et al., 2020) and the hESC line H9 (WA09) were used as negative control (Fig. 1C). Immunostaining was performed to assess the expression of the stemness markers Oct 4, Nanog and Sox2 (tested at passage 18, Fig. 1D). The expression of both cell surface (Tra-1–60 and SSEA4) and intracellular stemness markers (Nanog and Oct4) was also quantified by

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flow cytometry (tested at passage 18, Fig. 1E). The expression of ectoderm (OTX2), mesoderm (Brachyury) and endoderm (SOX17) markers upon differentiation demonstrated the ability of CUIMCi003-A to differentiate *in vitro* into the three germ-layers (tested at passage 18, Fig. 1F). Short tandem repeat (STR) analysis of the generated CUIMCi003-A line was performed through DNA fingerprint analysis (Cell Line Genetics, Madison, WI). The test revealed that all 15 hiPSC allele loci were consistent with those of the parental PBMCs (data available upon request). Mycoplasma test at passage 20 resulted negative (Supplementary Fig. 1). The *de novo* (p.P130A) *ARNT2* variant was confirmed in CUIMCi003-A by Sanger sequencing (Fig. 1G).

4. Materials and methods

4.1. iPSC maintenance

niPSCs were grown on feeders (γMEF, Life Technologies, cat #A34181) in DMEM/Ham's F-12 (Corning) supplemented with 20% KO-SR, 1x non-essential amino acids, 1x Penicillin-Streptomycin, 1x glutamine, 1x β-Mercaptoethanol (all Life Technologies) and 10 ng/ml FGF2 (R&D Systems). Cells were fed daily and split 1:6 when they reached 80% confluency using EDTA. Established iPSCs were maintained in feeder-free condition using Matrigel (Corning) coated plates and mTeSR1 or mTeSR Plus medium (Stem Cell Technologies). All cells were kept in an incubator at 37 °C, 5% CO_2, 20% O_2.

4.2. Karyotyping

CUIMCi003-A cells at passage 6 were examined by standard G-banding analysis (Cell Line Genetics, Madison, WI) on twenty-G banded metaphase cells at 450–500 band resolution.

4.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

At passage 17, 1×10^6 cells were collected and 1 μg of RNA was extracted using RNeasy Mini Kit (Qiagen, Cat # 74104). Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher, #4387406). The qRT-PCR was conducted on Applied Biosystems QuantStudio Flex 7 using the TaqMan® iPSC Sendai Detection Kit (ThermoFisher, #A13640). Primer sequences are listed in Table 2. PBMCs from the same cell line after Sendai transduction were used as a positive control for the expression of exogenous genes (=1 in the relative quantification). The iPSC line CUIMCi002-A (passage 32) and the hESC line H9 (WA09, passage 33) were used as negative control.

4.4. Immunostaining

At passage 18, iPSCs were fixed in 4% PFA (Santa Cruz Biotechnologies) for 10 min at room temperature (RT), washed twice with 1x PBS, then permeabilized for 30 min at RT with 0.1% Triton X-100. Cells were then incubated with Protein Block (Agilent Dako, Santa Clara, CA) for 30' at RT followed by primary antibodies for markers of stemness or differentiation (Table 2) in 1x PBS + 1% BSA over-night at RT. After washing twice, when required, cells were incubated with secondary antibody (Table 2) in 1x PBS + 1% BSA for 1hr at RT, followed by washing with 1x PBS + 1% BSA. Nuclei were stained with DAPI and

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cells visualized under an Olympus IX73 inverted microscope connected to a XM10 monochrome camera (Olympus, Tokyo, Japan).

4.5. Flow cytometry

Cell were dissociated by accutase treatment for 5 min at 37 °C, then fixed with 4% PFA for 15 min at RT. For intracellular staining, cells were permeabilized in methanol. Cells were then incubated for 15 min at RT with directly conjugated antibodies against OCT-3/4, NANOG, TRA-1–60 and SSEA4 (Table 2). Flow cytometry was performed on S3e (Bio-Rad). Data were analyzed using FlowJo™ Software (FlowJo 10.7.1. Ashland, OR: Becton, Dickinson and Company).

4.6. In vitro germ layer differentiation

To assess the 3-germ layer differentiation capability of CUIMCi003-A cells *in vitro,* the Human Pluripotent Stem Cell Functional Identification kit (R&D Systems, #SC027) was used according to manufacturer's instructions.

4.7. Mycoplasma detection

Absence of mycoplasma contamination was confirmed using e-Myco™ plus Mycoplasma PCR Detection Kit (Intron, Burlington, MA, #25234) according to manufacturer's instructions

4.8. Mutation analysis

Genomic DNA was extracted and purified from CUIMCi003-A cells by DNase blood and tissue kit (Qiagen, #69504). Genotyping of the heterozygous P130A variant in the *ARNT2* gene was performed by Sanger sequencing (Genewiz). Primers are listed in Table 2.

4.9. Short tandem repeat profiling

THA20_1 is the donor PBMC line for the iPSC line CUIMCi003-A. iPSC were tested at P17.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank the patients who generously donated their blood for the research conducted; Michael Kissner, Director of Operations, and the personnel of the Columbia Stem Cell Initiative Flow Core; Cecilia Sena, research coordinator.

Funding sources

This research was funded in part by Pilot and Feasibility Grant, New York Obesity and Nutrition Research Center, NIH-NIDDK, P30 DK026687-39, and Louis V. Gerstner Clinical Scholar Program at Columbia University Irving Medical Center.

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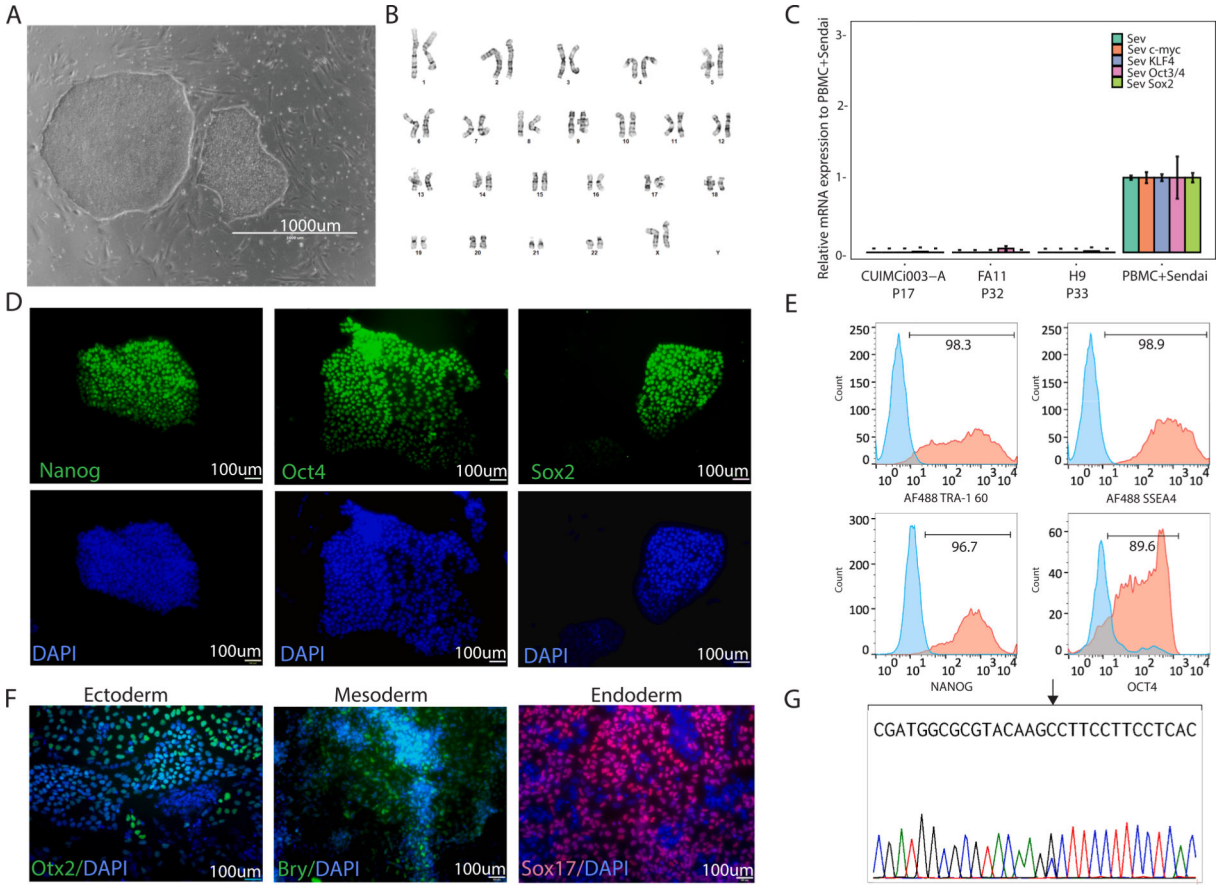

Fig. 1.
CUIMCi003-A characterization.

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Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Typical morphology	Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry)	Assess staining/expression of stemness markers at P18: Nanog, Oct4, Sox2	Fig. 1D
	Quantitative analysis (Flow cytometry, RT-qPCR)	Assess % of positive cells or transcripts for antigen & cell surface/intracellular markers at P18: Tra 1–60+: 98.3%, SSEA4+: 98.9%, Nanog+: 96.7%, Oct4+: 89.6% Sendai clearance assessment by qRT-PCR (at P17 for CUIMCi003-A; after transduction for the positive control)	Fig. 1E Fig. 1C
Genotype	Karyotype (G-banding) Resolution 450–500	46XX Resolution 450–500 at P6	Fig. 1B
Identity	STR analysis	DNA Profiling Performed 16 *loci* analyzed, all matching donor of origin at P17	Supplemental Table 1 available with authors
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous variant	Fig. 1G
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	PCR, Negative at P18	Supplemental Fig. 1
Differentiation potential	e.g. Embryoid body formation OR Directed differentiation	Proof of three germ-layers formation: positive OTX2 (ectoderm) staining, positive Brachyury (mesoderm) staining and positive SOX17 (endoderm) staining (at P18)	Fig. 1 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

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Table 2

Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Stemness Marker	Rabbit anti-OCT4 (Alexa Fluor 488 Conjugate)	1:50	Cell Signaling Technology Cat# 5177S, RRID: AB_10693303
Stemness Marker	Rabbit anti-NANOG	1:400	Cell Signaling Technology Cat# 4903P, RRID: AB_10559205
Stemness Marker	Mouse anti-SOX2 (Alexa Fluor 488 Conjugate)	1:50	Santa Cruz Biotechnology, Cat# sc-365823 RRID: AB_10842165
Stemness Marker (FACS)	Alexa Fluor 488 Mouse anti-Human TRA-1–60	1:20	BD Biosciences, Cat#560173
Stemness Marker (FACS)	Alexa Fluor 488 Mouse anti-SSEA-4	1:20	BD Biosciences, Cat#560308
Stemness Marker (FACS)	Alexa Fluor 488 Mouse anti-Human Nanog	1:20	BD Biosciences, Cat#560791
Stemness Marker (FACS)	Oct-4A - Rabbit mAb (Alexa Fluor 488 Conjugate)	1:20	Cell Signaling Technology Cat#5177
Stemness Marker – Isotype (FACS)	Alexa Fluor488 Mouse IgG1 κ Isotype Control	1:20	BD, Biosciences, Cat#557702
Stemness Marker – Isotype (FACS)	Rabbit mAb IgG - Isotype Control (Alexa Fluor488 Conjugate)	1:20	Cell Signaling Technology, Cat#2975S
Differentiation Markers	Goat anti-OTX2	1:20	R&D Systems Cat# AF1979, RRID: AB 2157172
Differentiation Markers	Goat anti-Brachyury	1:20	R&D Systems Cat# AF2085, RRID: AB_2200235
Differentiation Markers	Goat anti-SOX17 (NL557 Conjugate)	1:10	R&D Systems Cat# NL1924R, RRID: AB_2195645
Secondary antibody	Rabbit anti-Goat IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A27012, RRID: AB_2536077
Secondary antibody	Goat anti-Rabbit IgG Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A11008, RRID: AB_143165
Secondary antibody (FACS)	Goat anti mouse IgG1-PE	1:500	Molecular Probes Cat# P21129, RRID: AB_2539816
Secondary antibody (FACS)	Goat anti mouse IgM-488	1:500	Molecular Probes Cat# A21042, RRID: AB_141357
Primers qPCR	Target	Forward/Reverse primer (5′–3′)	
Sendai virus detection	*SeV*	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC	
Transgene detection	*KOS*	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG	
Transgene detection	*Klf4*	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA	
Transgene detection	*c-Myc*	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG	
Housekeeping gene	*18S*	Hs99999901s1, Applied Biosystem	
Primers mutational screening	Target	Forward/Reverse primer (5′–3′)	
ARNT2	*ARNT2*	GGTGTTAGCCCCTAGTTCCTGG TGGCTTCATTCCTTCCTCAACC	