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Lab Resource: Multiple Cell Lines

Generation of an induced pluripotent stem cell line (CIMAi001-A) from a compound heterozygous Primary Hyperoxaluria Type I (PH1) patient carrying p.G170R and p.R122* mutations in the AGXT gene.

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A B S T R A C T :

Primary Hyperoxaluria Type I (PH1) is a rare autosomal recessive metabolic disorder characterized by defects in enzymes involved in glyoxylate metabolism. PH1 is a life-threatening disease caused by the absence, deficiency or mistargeting of the hepatic alanine-glyoxylate aminotransferase (AGT) enzyme. A human induced pluripotent stem cell (iPSC) line was generated from dermal fibroblasts of a PH1 patient being compound heterozygous for the most common mutation c.508G>A (G170R), a mistargeting mutation, and c.364C>T (R122*), a previously reported nonsense mutation in AGTX. This iPSC line offers a useful resource to study the disease pathophysiology and a cell-based model for drug development.

Resource Table:

Unique stem cell line identifier	CIMAi001-A
Alternative name(s) of stem cell line	PH1-iPSC-G170R
Institution	Regenerative Medicine Program. CIMA Universidad de Navarra.
Contact information of distributor	Juan R. Rodriguez-Madoz (jrrodriguez@unav.es)
Type of cell line	iPSC
Origin	Human
Additional origin info	Age: 30 years
	Sex: Male
	Ethnicity: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Transgene free (CytoTune™-iPS 2.0 Sendai Reprogramming Kit)
Genetic Modification	YES
Type of Modification	Inherited
Associated disease	Primary hyperoxaluria type I
Gene/locus	AGXT c.508G>A (G170R) and c.364C>T (R122*)
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	26th July 2018
Cell line repository/ bank	N/A
Ethical approval	Patient informed consent obtained by the Ethical Committee of Clinical Investigation of Clinica Universidad de Navarra (Approval no. 171/2014)

Resource utility

This human induced pluripotent stem cell (iPSC) line is a useful tool for studies of disease phenotype and pathophysiology, and to be used as a cell-based disease model for drug development to treat PH1 patients.

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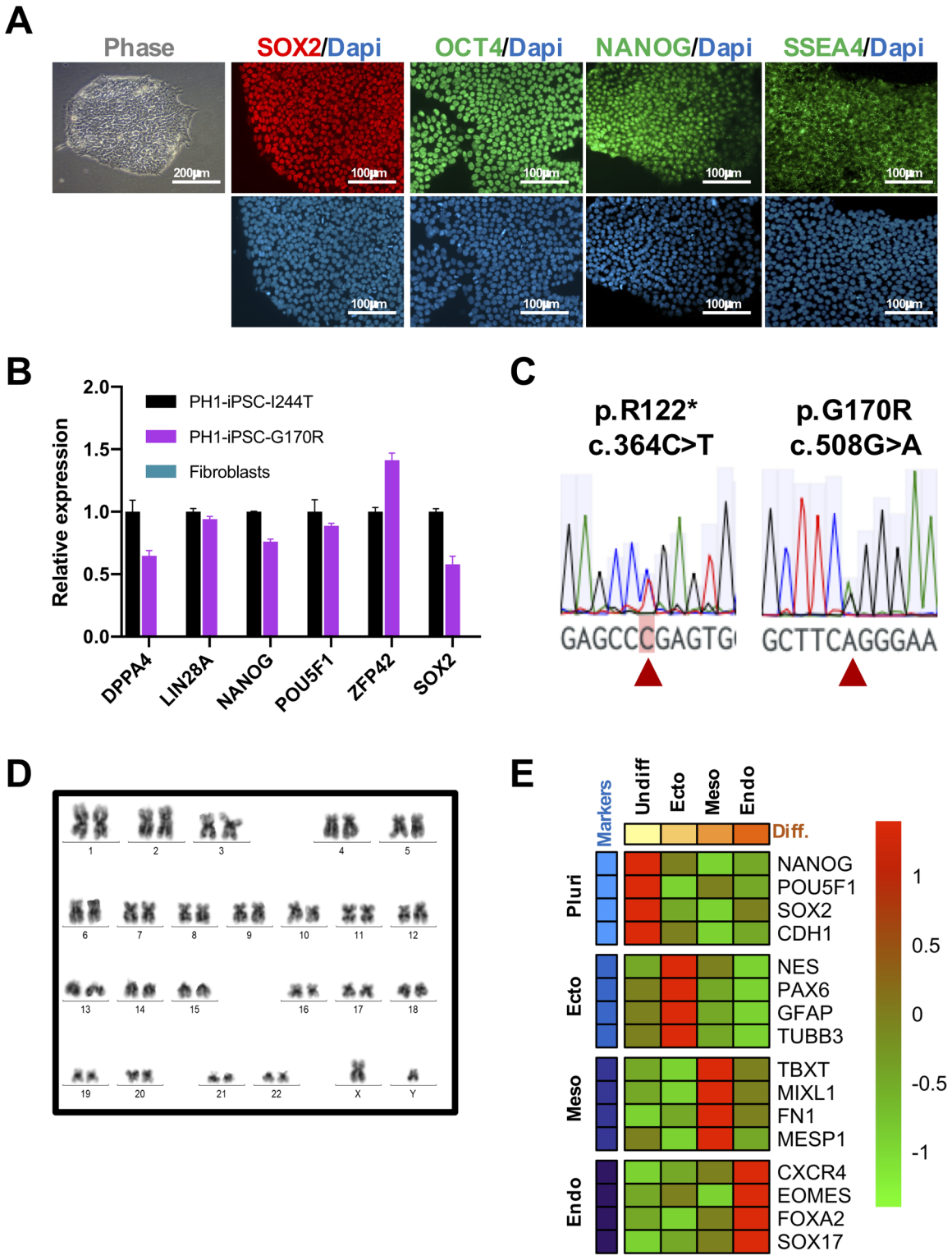

Figure 1. Characterization of PH1-iPSC-G170R line. (A) Typical round shape colony morphology with small, tightly packed cells and expression of pluripotency-associated markers (SOX2, OCT4, NANOG and SSEA4) by immunofluorescence. (B) Expression of endogenous pluripotency-associated markers DPPA4, LIN28A, NANOG, POU5F1, ZFP42 and SOX2 were confirmed by qPCR. (C) Genotyping of the PH1-iPSC-G170R line. Presence of the c.364C>T (R122*) and c.508G>A (G170R) mutations in AGXT gene were analyzed by sequencing. (D) Karyotype analysis of PH1-iPSC-G170R line depicting a normal 46XY karyotype. (E) Analysis if the differentiation capacity into three germ layers measured by qPCR using STEMdiff™ Trilineage Differentiation Kit (StemCell technologies).

Resource Details

Primary Hyperoxalurias (PH) are a group of rare autosomal recessive metabolic disorders characterized by defects in enzymes involved in glyoxylate metabolism. Primary hyperoxaluria type 1 (PH1), the most common form, is a life-threatening disease with an estimated prevalence of 1 to 3 cases per 1 million population, caused by the absence, deficiency or mistargeting of the hepatic alanine-

Table 1
Characterization and validation

Classification	Test	Result	Data
Morphology	Photography	Normal	Figure 1 panel A
Phenotype	Qualitative analysis: Immunofluorescence	SOX2, OCT4, NANOG, SSEA-4	Figure 1 panel A
	Quantitative analysis: RT-qPCR	DPPA4, LIN28A, NANOG, POU5F1 (OCT4), ZFP42 (REX1), SOX2	Figure 1 panel B
Genotype	Karyotype (G-banding) and resolution	Normal 46XY Resolution 400	Figure 1 panel D
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	16 sites tested; all sites matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Compound heterozygous mutation of AGXT p.R122*, p.G170R	Figure 1 panel C
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig. S1B
Differentiation potential	Directed differentiation (STEMdiff™ Trilineage Differentiation kit)	Confirmation of gene expression of ectodermal (NES, PAX6, GFAP, TUBB3), mesodermal (TBXT, MIXL1, FN1, MESP1) and endodermal (CXCR4, EOMES, FOXA2, SOX17) markers.	Figure 1 panel E
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-citometry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-NANOG	1:100	Abcam Cat# ab80892, RRID:AB_2150114
Pluripotency Markers	Rabbit anti-SOX2	1:500	Millipore Cat# AB5603, RRID:AB_2286686
Pluripotency Markers	Rabbit anti-OCT4	1:50	Santa Cruz Biotechnology Cat# sc-9081, RRID:AB_2167703
Pluripotency Markers	Mouse anti-SSEA4	1:1000	Cell signaling, Cat# 4755, RRID: AB_1264259
Secondary antibodies	Alexa Fluor 594 donkey anti-rabbit IgG	1:1000	Thermo Fisher, Cat# A21207, RRID: AB_141637
Secondary antibodies	Alexa Fluor 488 goat anti-rabbit IgG	1:200	Thermo Fisher, Cat# A11008, RRID: AB_143165
Secondary antibodies	Alexa Fluor 488 donkey anti-mouse IgG	1:200	Thermo Fisher, Cat# A21202, RRID: AB_141607

Primers

	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers	DPPA4	TGGTGTCAGGTGGTGTGTGG / CCAGGCTTGACCAGCATGAA
Pluripotency Markers	LIN28A	GGAGGCCAAGAAAGGGAATATGA / AACAATCTTGTGGCCACTTTGACA
Pluripotency Markers	NANOG	CCTGTGATTTGTGGGCCTG / GACAGTCTCCGTGTGAGGCAT
Pluripotency Markers	POU5F1	GGAAGGAATTGGGAACACAAAGG / AACTTCACCTTCCCTCCAACCA
Pluripotency Markers	ZFP42	TGGAGCCTGTGTGAACAGAA / CCACCTCCAGGCAGTAGTGA
Pluripotency Markers	SOX2	TGGCGAACCATCTCTGTGGT / CCAACGGTGTCAACCTGCAT
Pluripotency Markers	CDH1	GAGTGCCAACTGGACCATTC / ACCCACCTCTAAGGCCATCT
Ectodermal Markers	PAX6	CCCCACATATGCAGACACAC / TCACTTCCGGGAACTTGAAC
Ectodermal Markers	NES	CGTTGGAACAGAGGTTGGAG / GAGCGATCTGGCTCTGTAGG
Ectodermal Markers	GFAP	TGGAGGTTGAGAGGGACAAT / TAGGCAGCCAGGTTGTTCTC
Ectodermal Markers	TUBB3	GGCCTTTGGACATCTCTTCA / GACCGAATCCACCAGCTC
Mesodermal Markers	TBXT	ACTCACCTGCATGTTTATCCA / CCGTTGCTCACAGACCACAG
Mesodermal Markers	MIXL1	GGTACCCCGACATCCACTT / GCCAAAGGTTGGAAGGATTT
Mesodermal Markers	MESP1	GAAGGGCAGGCGATGGAG / CAGGCAGCCACTCCAGAG
Mesodermal Markers	FN1	CCCCATTCCAGGACACTTCT / TGCCTCCACTATGACGTTGT
Endodermal Markers	CXCR4	GGTGGACATTCATCTGTTTCC / CAAGACAAAAATCCAACAAGCA
Endodermal Markers	EOMES	GGCAAAGCCGACAATAACAT / TTCCCGAATGAAATCTCCTG
Endodermal Markers	FOXA2	CACTCGGCTTCCAGTATGCT / GTTCATGTTGCTCACGGAGG
Endodermal Markers	SOX17	GAATCCAGACCTGCACAACG / CTCTGCCTCCTCCACGAAG
House-Keeping Gene	GAPDH	CTGGTAAAGTGGATATTGTTGCCAT / TGGAATCATATTGGAACATGTAAACC
Mutation analysis	AGXT c.508G > A (p.G170R)	CTCAGCCTACCCGGAGTGTG /GGAGGGAAGTGGAGGGCATC
Mutation analysis	AGXT c.364C > T (p.R122*)	CACCCTCCTCTTCAGGCAGG /AGGACCAGGCTCCTGACTCA
Sev specific primers	SeV	GGATCACTAGGTGATATCGAGC / ACCAGACAAGAGTTTAAGAGATATGTATC
Sev specific primers	SeV_KOS	ATGCACCGCTACGACGTGAGCGC / ACCTTGACAATCCTGATGTGG
Sev specific primers	SeV_K	TTCCTGCATGCCAGAGGAGCCC / AATGTATCGAAGGTGCTCAA
Sev specific primers	SeV_M	TAACTGACTAGCAGGCTTGTC / TCCACATACAGTCCTGGATGATGATG

glyoxylate aminotransferase (AGT) enzyme (Cochat and Rumsby, 2013).

In this study we have generated an induced pluripotent stem cell (iPSC) line from dermal fibroblasts of a 30-year-old male primary hyperoxaluria type I (PH1)-diagnosed patient carrying compound heterozygous c.364C > T (R122*) and c.508G > A (G170R) mutations in AGXT gene. PH1-iPSC-G170R line was generated using the non-integrating CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Scientific, Invitrogen), which includes the reprogramming factors SOX2, POU5F1, cMYC and KLF4. This reprogramming system is based on a modified and non-transmissible form of Sendai virus (SeV) (Ban et al., 2011). PH1-iPSC-G170R line displayed a typical round shape ESC-like morphology with small and tightly packed cells, with a high nucleus/cytoplasm ratio and prominent nucleoli (Fig. 1A). The

presence of the c.508G > A and c.364C > T mutations in AGXT gene was confirmed (Fig. 1C) and the expression of several pluripotency-associated markers was corroborated by qPCR (Fig. 1B) and immunofluorescence (Fig. 1A). Moreover, the absence of exogenous reprogramming transgenes was observed by RT-PCR after 10 passages (Supplementary Fig. S1A). Differentiation capacity into three germ layers was demonstrated by trilineage differentiation (Fig. 1E). Finally, PH1-iPSC-G170R line showed normal karyotype (46, XY) (Fig. 1D), was not contaminated with mycoplasma (Supplementary Fig. S1) and cell line identity was corroborated using a short tandem repeat (STR) DNA analysis, which demonstrated matching genotypes at all 16 loci examined.

Materials and Methods

Ethical approval. All procedures were approved by the University of Navarra Ethical Committee and by the Advisory Committee for Human Tissue and Cell Donation and Use, according to Spanish and EU legislation. Fibroblast were obtained after written informed consent.

Cell culture. Fibroblasts were obtained from a skin biopsy by direct seeding of small tissue fragments under coverslips. Cells were cultured in gelatin-coated culture plates for a maximum of five passages before reprogramming in DMEM (Sigma) supplemented with 10% FBS (Gibco), 0.1 mM NEAA(Gibco), 2 mM L-glutamine (Lonza), and 100 UI/ml P/S (Lonza).

PH1-iPSC-G170R generation. Patient fibroblasts were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Scientific, Invitrogen). iPSCs were cultured at 37ºC and 5%CO_2 under feeder-free conditions using Matrigel™-coated culture dishes (BD Biosciences) and mTeSR™1 (StemCell Technologies). iPSCs were routinely passaged using ReLeSR™ (StemCell Technologies) at a splitting ratio of 1:3 or 1:6 every week when cells reached confluence (Fig. 1A).

Genomic DNA extraction and genotyping. Genomic DNA was isolated from PH1-iPSC-G170R and parental cells using NucleoSpin tissue kit (Macherey-Nagel). For genotyping 50 ng of DNA was amplified on a SimpliAmp™ Thermal Cycler (Applied Biosystems) using Platinum® Taq DNA Polymerase HF (Invitrogen) by specific primers (Table 2) with the following conditions: 95ºC 2', [95ºC 20'', 63ºC 20'', 72ºC 40'']x40, 72ºC 3'. The presence of the c.364C > T (R122*) and c.508G > A (G170R) mutations in AGXT gene were analyzed in the PCR purified products (456bp and 751bp respectively) by sequencing (Fig. 1C).

Trilineage differentiation. Directed differentiation into three germ layers was achieved using STEMdiff™ Trilineage Differentiation Kit (StemCell technologies) according to manufacturer's instructions. In brief, PH1-iPSC-G170R line was seeded at recommended cell densities onto Matrigel™-coated culture dishes (BD Biosciences). Cells were cultured in lineage specific medium with daily medium changes until day 5 for meso- and endodermal and until day 7 for ectodermal differentiation, respectively. Expression of lineage specific markers was performed by qPCR as described below.

RNA extraction and RT-qPCR. Total RNA was isolated with Maxwell® 16 LEV simplyRNA Tissue Kit (Promega). RNA concentration was determined using a NanoDrop (Thermo Scientific) and RNA quality tested using Bioanalyzer (Agilent). cDNA was synthesized using PrimeScript™-RT reagent Kit (Takara). qPCR primers (Table 2) were designed using Primer3 input software. Expression of pluripotency-associated and linage-specific markers was evaluated in 10ng of cDNA by qPCR using PowerUp™ SYBR® Green Master Mix (Applied Biosystems) on a QuantStudio 5 Real-Time PCR System (Applied Biosystems) with the following parameters: 50ºC 2', 95ºC 10', [95ºC 15'', 60ºC 1']x40, melting curve 60ºC-95ºC. Data was analyzed using delta-CT method. Gene expression levels were normalized to GAPDH expression (Fig. 1B). Silencing of the exogenous reprogramming factors was analyzed following CytoTune™-iPS 2.0 manufacturer's instructions (Supplementary Fig. S1A).

Immunofluorescence (IF). IF was performed as described (Zapata-Linares et al., 2016). Briefly, PH1-iPSC-G170R was fixed with 4% PFA (Sigma), permeabilized for 10 minutes with 1% TritonX-100 (Sigma) in PBS and blocked with 5% BSA for 30 minutes. SOX2 (Millipore), POU5F1 (Santa Cruz), NANOG (Abcam) and SSEA-4 (Cell Signalling) primary antibodies were diluted in PBS/TBS with 1% BSA and incubated for one hour at RT. Alexa Flour-488- and Alexa Fluor-594-conjugated secondary antibodies (Thermo Fisher) were incubated for 1-1.5 hours at RT. Samples were visualized under an inverted fluorescence microscope (Nikon Eclipse Ti-S) (Fig. 1A). Antibodies used and dilutions are listed in Table 2.

Karyotype analysis. Chromosomal analysis was performed on PH1-iPSC-G170R line at passage 20-22 by GTG-banding analysis at 400 bands of resolution on 20 metaphase spreads at the Molecular Cytogenetics Group, Spanish National Cancer Centre-CNIO, according to the International System Cytogenetics Nomenclature recommendations.

Short tandem repeat (STR) analysis. STR analysis of PH1-iPSC-G170R line and its parenteral fibroblast was performed at CIMA LAB Diagnostics using AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems). Multiplex PCR performed for the STRs D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, AMEL, D5S818 and FGA confirmed cell identity.

Mycoplasma detection. Mycoplasma testing was performed and analyzed using the Lonza MycoAlert kit. Ratio B/A > 1.2 indicates mycoplasma positive. Ratio B/A 0.9–1.2 indicates ambiguous results and Ratio B/A < 0.9 indicates mycoplasma negative (Supplementary Fig. S1B).

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101626.

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