### Title

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### Publication Date

2023-06-01

### DOI

10.1016/j.scr.2023.103117

Peer reviewed

Published in final edited form as:

*Stem Cell Res*. 2023 June ; 69: 103117. doi:10.1016/j.scr.2023.103117.

# Generation of two induced pluripotent stem cell lines (CHOCi002-A and CHOCi003-A) from Pompe disease patients with compound heterozygous mutations in the *GAA* gene

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## Abstract

Pompe disease is an autosomal recessive lysosomal storage disease caused by pathogenic variants in *GAA*, which encodes an enzyme integral to glycogen catabolism, acid α-glucosidase. Disease-relevant cell lines are necessary to evaluate the efficacy of genotype-specific therapies. Dermal fibroblasts from two patients presenting clinically with Pompe disease were reprogrammed to induced pluripotent stem cells using the Sendai viral method. One patient is compound heterozygous for the c.258dupC (p.N87QfsX9) frameshift mutation and the c.2227C>T (p.Q743X) nonsense mutation. The other patient harbors the c.−32–13T>G splice variant and the c.1826dupA (p.Y609X) frameshift mutation in compound heterozygosity.

### Keywords

Pompe disease; Lysosomal storage disorder; Induced pluripotent stem cells; Sendai virus



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CRediT authorship contribution statement

**Chloe Christensen:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Perla Heckman:** Investigation. **Allisandra Rha:** Methodology. **Shih-Hsin Kan:** Resources, Writing – review & editing. **Jerry Harb:** Writing – review & editing. **Raymond Wang:** Supervision, Project administration, Funding acquisition, Writing – review & editing.

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.2023.103117.

Resource Table

| | |
|---|---|
| **Unique stem cell lines identifier** | 1: CHOCi002-A |
| | 2: CHOCi003-A |
| **Alternative name(s) of stem cell lines** | N/A |
| **Institution** | CHOC Children's Research Institute |
| **Contact information of distributor** | Chloe Christensen, chloe.christensen@choc.org |
| **Type of cell lines** | iPSC |
| **Origin** | Human |
| **Additional origin info** | Age: 7.7 y, 16.1 y |
| | Sex: F, M |
| | Ethnicity: Caucasian, Caucasian |
| **Cell source** | Dermal fibroblasts |
| **Clonality** | Clonal |
| **Method of reprogramming** | Non-integrating Sendai virus |
| **Genetic modification** | Yes |
| **Type of genetic modification** | Hereditary |
| **Evidence of the reprogramming transgene loss** | RT-PCR |
| **Microbiology and virology** | Mycoplasma testing by luminescence: Negative |
| **Associated disease** | Infantile-onset Pompe disease, Late-onset Pompe disease |
| **Gene/locus** | 1: Chromosome 17q25.2-q25.3, *GAA*: [c.258dupC];[c.2227C>T] |
| | 2: Chromosome 17q25.2-q25.3, *GAA*:[c.−32–13T>G];[c.1826dupA] |
| **Date archived/stock date** | April 29, 2022 |
| **Cell line repository/bank** | https://hpscreg.eu/cell-line/CHOCi002-A<br>https://hpscreg.eu/cell-line/CHOCi003-A |
| **Ethical approval** | Ethics approval was obtained from Children's Hospital of Orange County Institutional Review Board, #130990. Informed consent was obtained for biopsy collection. |

## 1. Resource utility

The human induced pluripotent stem cell (iPSC) lines reported here harbor pathogenic mutations associated with infantile and late-onset Pompe disease (PD) (de Faria et al., 2021). These iPSC lines allow for further elucidation of PD cellular phenotype and for the study of genome editing-based therapeutic strategies that target patient-specific genotypes.

## 2. Resource details

PD is a rare lysosomal storage disease with a prevalence of 1:23,000 (Park, 2021). Pathogenic mutations in *GAA* render acid α-glucosidase non-functional, impairing glycogen metabolism. Without intervention, patients experience hypertrophic cardiomyopathy and muscle weakness and die within the first year of life. The iPSC lines that exist for some common *GAA* mutations are useful resources for elucidating disease mechanisms and assessing therapeutic strategies *in vitro* (Cheng et al., 2019; van der Wal et al., 2017). However, there are more than 700 known pathogenic mutations causative for PD, the vast

majority of which are not represented in existing iPSC lines (de Faria et al., 2021). To our knowledge, the PD iPSC lines described here are the first reported with these genotypes, and all mutations except the *GAA* c.−32–13T>G pathogenic variant are not present in existing PD iPSC models (van der Wal et al., 2017). Cellular models of PD generated from these iPSCs will be invaluable for the evaluation of gene therapies that target patient-specific mutations.

The two iPSC lines described here (CHOCi002-A and CHOCi003-A) were generated using the CytoTune™-iPS Sendai reprogramming method (see Table 1). Dermal fibroblasts derived from a patient with the *GAA* c.258dupC (p.N87QfsX9) frameshift mutation and the c.2227C>T (p.Q743X) nonsense mutation present in compound heterozygosity were used to generate CHOCi002-A. Dermal fibroblasts derived from a second patient with the c.−32–13T>G splice variant and the c.1826dupA (p.Y609X) frameshift mutation in compound heterozygosity were used to generate CHOCi003-A. Sanger sequencing confirmed that both cell lines maintained their *GAA* genotype post-reprogramming (Fig. 1A). RT-qPCR analysis showed that both iPSC lines have gene expression consistent with downregulation of genes associated with ecto-, meso-, and endoderm, and similar self-renewal gene expression when compared to a pluripotent reference standard (Fig. 1B). Of note, RT-qPCR analysis assessed 85 genes including *SOX2, NANOG, POU5F1* (*OCT3/4*), *HAND1, SOX17, GATA4, and LEFTY1* (Fig. 1B).

Monoclonal colonies exhibiting the morphological hallmarks of iPSCs were isolated and expanded (Fig. 1C). Pluripotency was further evidenced through immunofluorescence assessment of pluripotency markers OCT3/4, SOX2, SSEA-4, TRA-1–60, and TRA-1–81 (Fig. 1D). Karyotyping revealed no chromosomal abnormalities (Fig. 1E). RT-PCR for pluripotency markers showed elevated *SOX2* and *NANOG* expression levels in iPSCs compared to parental fibroblasts (Fig. 1F). Measurements of *OCT4* expression showed an increase in comparison to parental fibroblasts for CHOCi002-A but were inconclusive for CHOCi003-A (Fig. 1F). However, RT-qPCR-based measurement of *POU5F1* (*OCT3/4*) expression in CHOCi003-A showed levels consistent with a pluripotent reference standard (Fig. 1B). Transgene loss was confirmed through RT-PCR with Sendai virus, KOS, Klf4, and c-Myc-specific primers (Fig. 1G). Differentiation potential was confirmed through directed differentiation followed by immunofluorescence assessment for expression of markers for ectoderm (OTX2/SOX1), mesoderm (Brachyury/HAND1), and endoderm (GATA4/SOX17) (Fig. 1H). Short tandem repeat (STR) analysis confirmed common identity between iPSCs and parental fibroblasts at 17 genomic loci (submitted to journal). Both cell lines were confirmed to be mycoplasma free (Suppl. Table S1). In conclusion, CHOCi002-A and CHOCi003-A demonstrate the pluripotency characteristics necessary for future use in genome editing strategy testing and disease modelling.

## 3. Materials and methods

### 3.1. Reprogramming of human dermal fibroblasts

Biopsied tissue from two donor patients was mechanically disassociated and cultured in high glucose Dulbecco's Modified Eagle Medium (Cytiva) supplemented with 20% fetal bovine serum (Omega), 1% non-essential amino acids (Gibco), and 37.5 μg/mL Primocin

(Invivogen). Fibroblasts (passage (P)6, CHOCi002-A; P11, CHOCi003-A) were transduced using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) with a MOI of 10:10:6. On day 7 posttransduction, cells were passaged to Vitronectin-coated plates (Gibco; 0.5 μg/cm$^2$). On day 8, media was switched to Essential 8™ (Gibco) and replenished everyday thereafter. Emerging stem cell colonies were mechanically picked and expanded. Cells were passaged using UltraPure™ EDTA (Invitrogen, 0.5 mM) and were supplemented with 10 μM Y-27632 (STEMCELL Technologies) for 24 h after passaging or thawing.

### 3.2. Genomic DNA isolation and genotyping

Genomic DNA was isolated using QuickExtract™ DNA Extraction Solution (Biosearch Technologies) as per the manufacturer's protocol. *GAA* exons 2, 13 and 16 were amplified by PCR. The genotypes were confirmed through Sanger sequencing. Primers are listed in Table 2.

### 3.3. Real Time-qPCR and karyotyping

RT-qPCR and karyotyping were performed by Thermo Fisher Scientific Stem Cell Characterization Services. Cell pellets (>2 × 10$^6$ cells; P10) were prepared according to the Genomic DNA Purification Kit or PureLink™ RNA Mini Kit. DNase-treated RNA was reverse transcribed using the high-capacity cDNA Reverse Transcription Kit with RNase inhibitor. cDNA samples were prepared for RT-qPCR using the TaqMan® hPSC Scorecard™ Kit. Total gDNA (100 ng) was used to prepare the Cytoscan HT-CMA 96 array for KaryoStat+.

### 3.4. Reverse Transcriptase-PCR

Cell pellets (5x10$^5$ cells; P1 and 16, CHOCi002-A; P12, CHOCi003-A) were treated with TRIzol reagent (Invitrogen) and RNA was isolated using the Direct-zol™ RNA MiniPrep Kit (Zymo Research). RNA was reverse transcribed using the iScript™ cDNA synthesis Kit (Bio-Rad) according to the manufacturer's protocol. cDNA was amplified using site-specific PCR primers.

### 3.5. Immunofluorescence

*Pluripotency immunofluorescence*: iPSCs (P16-26) were fixed in 4% formaldehyde, permeabilized in 0.5% Triton® X-100 (for non-surface markers) and blocked in 3% bovine serum albumin. Cells were incubated with primary antibodies at 4 °C overnight. Cells were incubated with secondary antibodies at room temperature for one hour. *Trilineage immunofluorescence*: iPSCs (P10-26) were differentiated using either the StemMACS™ Trilineage Differentiation Kit, human, (Miltenyi Biotec) or to cardiac mesoderm using the PSC Cardiomyocyte Differentiation Kit (Gibco). Cells were processed for immunocytochemistry using the Human Three Germ Layer 3-Color Immunocytochemistry Kit (R&D Systems) and Alexa Fluor 488-conjugated secondary antibodies were applied where indicated. Slides were mounted in VECTASHIELD® Antifade Mounting Media with DAPI (Vector Laboratories). Cell imaging was performed using a Keyence BZ-X810 fluorescence microscope with BZ-X800 Viewer software.

### 3.6. Short tandem repeat analysis

STR analysis was performed by the ATCC Human Cell Authentication Service. Cells ($1x10^6$) were spotted on FTA™ paper (135-XV™). Amplification of 17 STR loci was performed using a PowerPlex® 18D System (Promega). Data were processed using GeneMapper™ ID-X 1.6 software (Applied Biosystems™).

### 3.7. Mycoplasma assessment

Absence of mycoplasma (at P5) was confirmed using the MycoAlert Mycoplasma Detection Kit (Lonza) according to the manufacturer's protocol.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

This work was supported by the Campbell Foundation of Caring, the CHOC Foundation (One Wish, 2019), the Larry and Helen Hoag Foundation, and the National Institutes of Health (1-R01AR079223-01, 2021).

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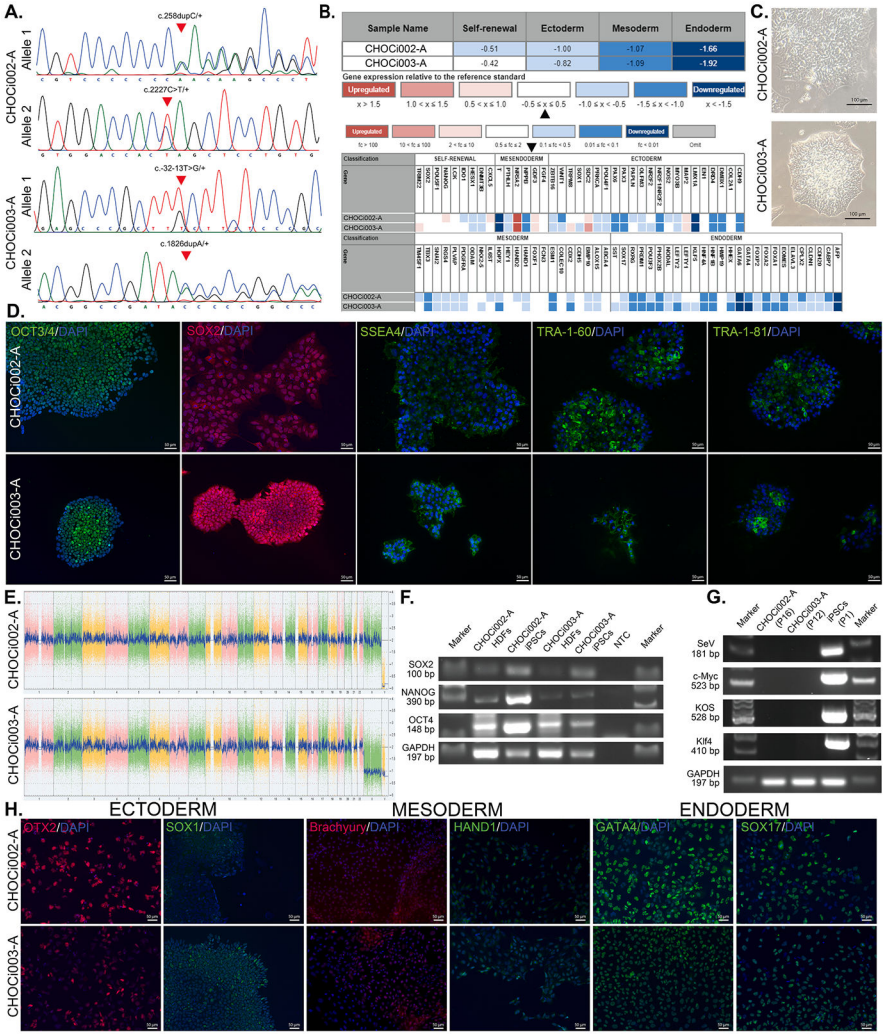


**Fig. 1.**

Characterization and validation of CHOCi002-A and CHOCi003-A iPSC lines.

**Table 1**

Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| **Morphology** | Photography Bright-field | Normal | Fig. 1C |
| **Phenotype** | Qualitative analysis (immunocytochemistry, RT-PCR) | Expression of pluripotency markers (TRA-1–81, TRA-1–60, SOX2, SSEA-4, OCT3/4) assessed by immunocytochemistry; Expression of *NANOG, SOX2, OCT4*, assessed by RT-PCR | Fig. 1D, F |
| | Quantitative analysis (TaqMan® qRT-PCR) | Scored positively for self-renewal markers, shows downregulation for markers of ectoderm, mesoderm, and endoderm | Fig. 1B |
| **Genotype** | Karyotype (microarray) | 46XX, 46XY; No chromosomal aberrations<br>Resolution: detection of > 1 Mb for chromosomal gains and losses | Fig. 1E |
| **Identity** | Microsatellite PCR (mPCR) OR STR analysis | N/A | N/A |
| | | 17/17 loci matched | Submitted in archive with journal |
| **Mutation analysis** | Sequencing | Compound heterozygous duplication (c.258dupC) and nonsense variant (c.2227C>T); Heterozygous intronic splice variant (c.−32–13T>G) and duplication (c.1826dupA) | Fig. 1A |
| | Southern Blot OR WGS | N/A | N/A |
| **Microbiology and virology** | Mycoplasma | Mycoplasma testing by luminescence: Negative | Supplementary Table S1 |
| **Differentiation potential** | Directed differentiation | Ectoderm, endoderm and mesoderm formation. Expression of OTX2 and SOX1, GATA4 and SOX17, and Brachyury and HAND1, respectively | Fig. 1H |
| **Donor screening** | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A | N/A |
| **Genotype additional info** | Blood group genotyping | N/A | N/A |
| | HLA tissue typing | N/A | N/A |

**Table 2**

Reagents details.

| | **Antibodies used for immunocytochemistry** | | | |
|---|---|---|---|---|
| | **Antibody** | **Dilution** | **Company Cat #** | **RRID** |
| **Pluripotency markers** | Mouse anti-Oct3/4 | 1:100 | Santa Cruz Biotechnology Cat# SC-5279 | RRID:AB_628051 |
| | Rabbit anti-SOX2 | 1:50 | Invitrogen Cat# A24339 | RRID:AB_2924437 |
| | Mouse anti-SSEA-4 | 1:100 | BD Biosciences Cat# 560308 | RRID:AB_1645371 |
| | Mouse anti-TRA-1–60 | 1:100 | Millipore Sigma Cat# MAB4360 | RRID:AB_2119183 |
| | Mouse anti-TRA-1–81 | 1:10 | BD Biosciences Cat# 560461 | RRID:AB_2869349 |
| **Differentiation markers** | Goat anti-OTX2 | 1:10 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| | Goat anti-SOX1 | 1:10 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| | Goat anti-Brachyury | 1:10 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| | Goat anti-HAND1 | 1:10 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| | Goat anti-GATA-4 | 1:10 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| | Goat anti-SOX17 | 1:10 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| **Secondary antibodies** | Alexa Fluor 488 Goat Anti-Mouse IgG (H + L) | 1:500 | Southern Biotech Cat# 1036-30 | RRID:AB_2794351 |
| | Alexa Fluor 488 Goat Anti-Mouse IgM | 1:500 | Southern Biotech Cat# 1021-30 | RRID:AB_2794251 |
| | Alexa Fluor 488 Donkey Anti-Goat IgG | 1:500 | Invitrogen Cat# A11055 | RRID:AB_2534102 |
| | Alexa Fluor 594 Donkey Anti-Rabbit | 1:250 | R&D Systems Cat# SC022 | RRID:AB_2889887 |
| | **Primers** | | | |
| | **Target** | **Size of band** | **Forward/Reverse primer (5′ −3′)** | |
| **SeV specific primers (RT-PCR)** | *SeV* | 181 bp | GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC | |
| | *c-Myc* | 523 bp | TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG | |
| | *KOS* | 528 bp | ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG | |
| | *Klf4* | 410 bp | TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA | |
| **Pluripotency markers (RT-PCR)** | *SOX2* | 100 bp | GCTTAGCCTCGTCGATGAAC/AACCCCAAGATGCACAACTC | |
| | *NANOG* | 390 bp | CAGCCCCGATTCTTCCACCAGTCCC/CGGAAGATTCCCAGTCGGGTTCACC | |
| | *OCT4* | 148 bp | GACAGGGGGAGGGGAGGAGCTAGG/CTTCCCTCCAACCAGTTGCCCCAAAC | |
| **Housekeeping gene (RT-PCR)** | *GAPDH* | 197 bp | GGAGCGAGATCCCTCCAAAAT/GGCTGTTGTCATACTTCTCATGG | |
| **Genotyping** | *GAA*, Exon 2 | 665 bp | GTGTCTACCTGCCTTGCTGG/TGCTTTGCAGGGATGTAGCAA | |
| | | 551 bp | GATGTCTCAGAGCTGCTTTG/CCATTTCAGAGGAGCTCAGGTT | |

| | | |
|---|---|---|
| *GAA*, Exon 13 | 703 bp | CAGACAGAGGCAACTGTGCC/CTTCTCCAGCAGGGGTGGGATT |
| *GAA*, Exon 16 | 518 bp | AGTCCTCCAAGTCCTCCGGCAC/GCAGAACCTGGCCAAGTCCCAC |