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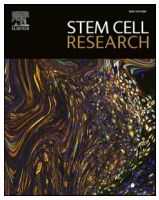

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line from a patient with GM3 synthase deficiency using self-replicating RNA vector

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A B S T R A C T

GM3 synthase deficiency (GM3SD) is caused by biallelic variants in the *ST3GAL5* gene. Early clinical features of GM3SD include infantile onset of severe irritability and feeding difficulties, early intractable seizures, growth failure, hypotonia, sensorineural hearing impairment. We describe the generation and characterization the human induced pluripotent stem cell (hiPSC) line derived from fibroblasts of a 13-year-old girl with GM3 synthase deficiency resulted compound heterozygous for two new variants in the *ST3GAL5* gene, c.1166A > G (p.His389Arg) and the c.1024G > A (p.Gly342Ser). The generated hiPSC line shows a normal karyotype, expresses pluripotency markers, and is able to differentiate into the three germ layers.

Resource Table

Unique stem cell line identifier	AOUMEYi002-A
Alternative name(s) of stem cell lines	N/A
Institution	Neuroscience Department, Meyer Children's Hospital IRCCS, Florence, Italy.
Contact information of distributor	Rodolfo Tonin – *rodolfo.tonin@meyer.it* Amelia Morrone – *amelia.morrone@meyer.it*
Type of cell lines	iPSC
Origin	Human
Additional origin info required	AOUMEYi002-A Age: 13 years Sex: F Ethnicity: Central European
Cell Source	Skin fibroblasts
Clonality	Mixed
Associated disease	GM3 synthase deficiency
Gene/locus	*ST3GAL5*; GRCh38: Chr 2:85,837,120–85,889,034
Date archived/stock date	N/A
Cell line repository/ bank	https://hpscreg.eu/user/cellline/edit/AOUMEYi002-A
Ethical approval	The study was approved by the Pediatric Ethics Committee of the Tuscany Region, Italy (No 283/2021). Written informed consent was obtained from patient's guardians.

(*continued on next column*)

Resource Table (*continued*)

1. Resource utility

Patients with GM3 synthase deficiency (GM3SD) manifest a severe clinical picture characterized by early onset and dramatic neurological and cognitive impairment. The generated hiPSC line, obtained by reprogramming the GM3SD patient's somatic cells, will be instrumental in studying the pathophysiological mechanisms of the disease in a specific cell type and in a patient-specific contest. Table 1

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis by Immunocytochemistry	hiPCS line expresses pluripotency markers: OCT4, SOX2 and NANOG.	Fig. 1 panel A
	Quantitative analysis by RT-qPCR	hiPCS line expresses stemness markers: *OCT4, SOX2, KLF4*	Fig. 1 Panel B
Genotype	Karyotype	AOUMEYi002-A: 46, XX hiPCS line expresses normal karyotype.	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	16 markers tested- matched	Fig. 1 Panel E; Supplementary Fig. 1
Mutation analysis	DNA Fragment Analysis by Capillary Electrophoresis	hiPSC line is characterized; it is heterozygous for 2 different variants in *ST3GAL5* gene	Fig. 1 Panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma detection by Luminescence	Negative	Supplementary Fig.2
Differentiation potential	Directed differentiation	Expression of layer-specific markers: Ectoderm (SOX1), Mesoderm (BRACHYURY, EOMES) and Endoderm (SOX17, GATA4).	Fig. 1 panel E

2. Resource details

Loss of GM3 biosynthesis in GM3 synthase deficiency is associated with progressive microcephaly, due to impaired neurogenesis, dyskinetic movements, seizures, hearing loss, and variable defects in skin pigmentation (Rudy et al., 2022). The neuronal manifestations of GM3SD demonstrate the essential role of ganglioside-mediated processes during neurogenesis, and proper functional maintenance of neuronal cell types after development (Trinchera et al., 2018).

After molecular testing, we took skin punch biopsies from a 13-year-old girl diagnosed with GM3SD. The patient was compound heterozygous for two novel variants in the *ST3GAL5* gene: c.1166A > G; p.His389Arg, inherited from her mother, and c.1024G > A; p.Gly342Ser, which occurred *de novo*.

Previous clinical history revealed at two months of age, onset of an epileptic encephalopathy with infantile spasms, acquired microcephaly, severe developmental delay, dyskinetic quadriparesis and bilateral sensorineural hearing loss. Brain MRI at the age of seven revealed mild cortico-subcortical atrophy with minor white matter hypomyelination in the temporopolar regions, and unrevealing spectroscopy.

We reprogrammed fibroblasts using a non-integrating, self-replicating RNA reprogramming vector. The generated iPSC clones presented with a typical stem cell morphology with tightly packed colonies and a high nuclear/cytoplasmic ratio (bright-field image in Fig. 1A). Cytogenetic analysis confirmed a normal female karyotype (46, XX) without structural chromosomal abnormalities (Fig. 1C). We assessed nuclear pluripotency markers through immunocytochemistry analysis (Fig. 1A). In contrast to fibroblasts from the same patient that were not reprogrammed, the iPSC clones expressed the canonical stemness markers OCT3/4, SOX2 and KLF4 (Fig. 1B). We confirmed the genotype of the disease-causing mutations in the generated iPSC colonies by NGS, and the identity of these lines was also confirmed by STR analysis (Fig. 1E and Supplementary Data 1).

To assess the pluripotency and the differentiation potential of the generated iPSC line, we performed directed differentiation of iPSCs in all three germ layers (ectoderm, endoderm and mesoderm). Immunocytochemistry performed to characterise specific differentiation markers for each layer confirmed the identity of the differentiated cells (Fig. 1D). AOUMEYi002-A line was validated for clearance of residual self-replicating RNA vector and resulted vector-free. All cell lines were free of mycoplasma contamination (Supplementary Data 2).

Our results confirm the successful generation of an iPSC line with *ST3GAL5* gene mutations. This line will prove useful in further studies aimed at clarifying the pathophysiology of mutations in GM3SD. The line will also be helpful for drug screening and development.

3. Materials and methods

3.1. Skin biopsy and isolation of patient's fibroblasts

We collected skin biopsies using a dermal puncher tool from the patient's forearm and were cultured for two weeks with Dulbecco's modified Eagle's medium (DMEM-Ref: 41965 – 035 Gibco) supplemented with fetal bovine serum (10 %), 2 mM of L-glutamine and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin) to obtain a primary culture of fibroblasts. Written informed consent had been obtained. The sample was anonymized and used only for research purposes.

3.2. iPSC Reprogramming and cell culture

We reprogrammed human fibroblasts at passage 3 using ReproRNA™-OKSGM Kit (Catalog # 05930, Stemcell Technologies) according to the manufacturer's guidelines. 17–18 days after transfection, iPSC colonies could be manually picked. We cultured iPSCs on plates coated with Corning® Matrigel® hESC-Qualified Matrix (#CLS354277-1EA, Corning) or Vitronectin XF™ (Catalog # 07180, Stemcell Technologies) in mTeSR™ Plus medium (Catalog # 100–0276, StemCell) and passaged manually with PBS-EDTA 0,5 mM with a splitting ratio 1:8 every 4–5 days without ROCK inhibitor. Cells were maintained in a humidified atmosphere at 37 °C with 5 % CO_2.

3.3. RT-qPCR

We isolated total RNA from iPSC clones and fibroblasts with RNeasy Mini Kit (50) (Catalog no. #74104, QIAGEN). Gene expression levels of pluripotency markers were validated by qRT-PCR using QuantiNova SYBR Green RT-PCR Kit (Catalog #208152, QIAGEN) on the Applied Biosystems 7500. Expression data were normalized to the housekeeping gene *PBGD* using the $2^{-\Delta\Delta CT}$ method. Oligo primers used for qRT-PCR are listed in Table 2.

3.4. Immunocytochemistry

Cells grown on Matrigel-coated coverslips at passage numbers 10–15 were fixed with 4 % paraformaldehyde (PFA) for 10 min at 37 °C, permeabilized using 0.1 % Triton X-100 in PBS for 15 min and blocked with 3 % bovine serum albumin and 0.01 % Triton X-100 in PBS. Then, cells were incubated overnight at 4 °C with primary antibodies listed in Table 2. These antibodies were detected via secondary antibody staining

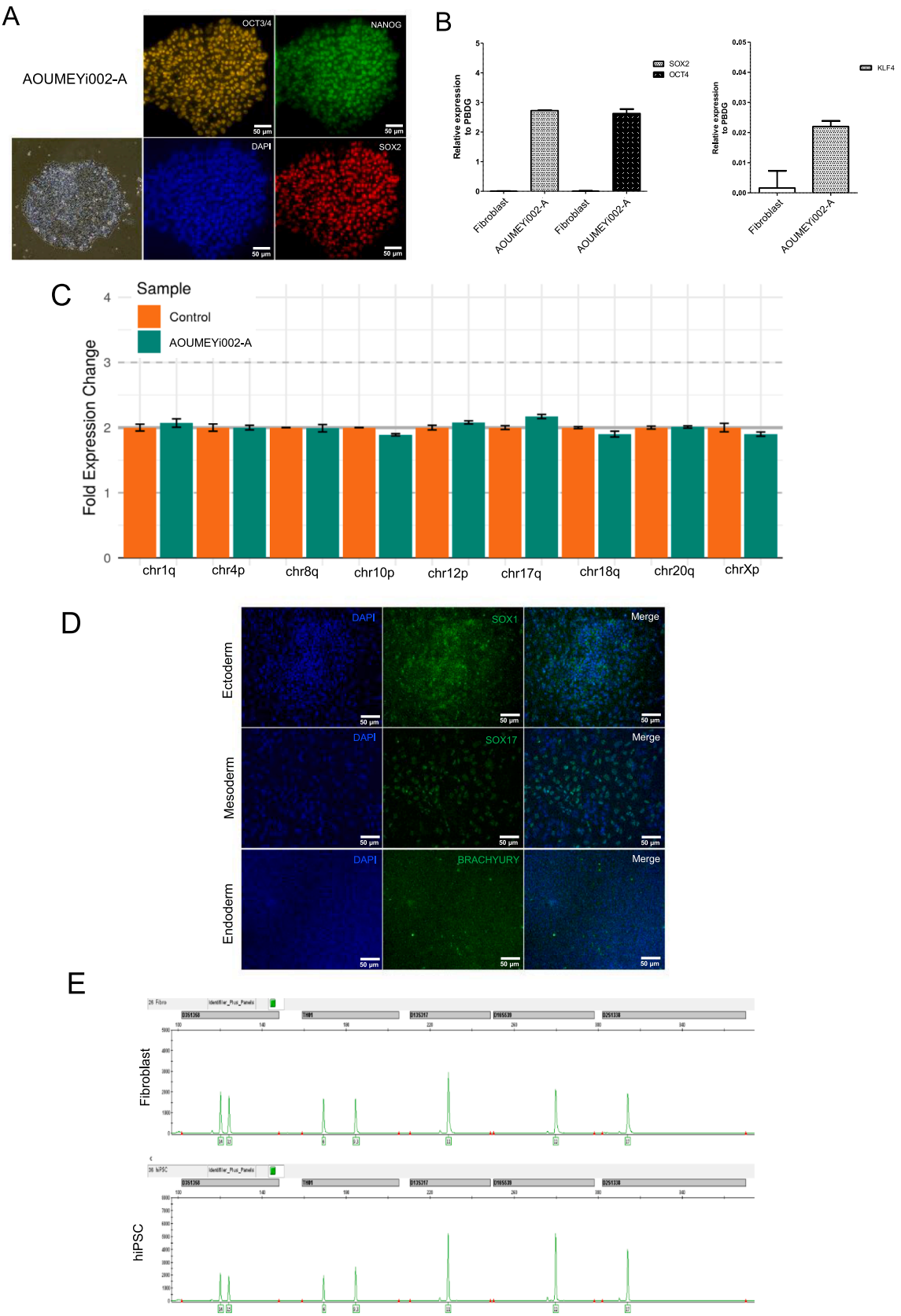

Fig. 1. Characterization of AOUMEYi002-A iPSC line.

Table 2
Reagents details Antibodies and stains used for immunocytochemistry.

	Antibody	Dilution	Company Cat #
Pluripotency Markers	PE Mouse anti-OCT3/4 IgG2b	1:100	Stemcell Technologies Cat # 60093PE, Clone 3A2A20, PE
	Mouse anti-SOX2 IgG2A	1:100	R&D Systems Cat # MAB2018
	Goat anti-Nanog IgG	1:100	R&D Systems Cat # AF1997
Differentiation Markers	Goat anti-SOX17 IgG	1:200	R&D Systems Cat # AF1924
	Biotinylated Goat anti-GATA-4 IgG	1:100	R&D Systems Cat # BAF2606
	Goat anti-SOX1	1:200	R&D Systems Cat # AF3369
	Goat anti-Brachyury IgG	1:100	R&D Systems Cat # AF2085
Secondary antibodies	Alexa Fluor 647 Goat anti-mouse IgG (H + L), F(ab') 2 Fragment	1:1000	Cell Signaling Cat#BK4410S
	NorthernLights™493 Donkey Anti-goat IgG (H + L)	1:500	R&D Systems Cat #NL003
	NorthernLights™ 557 Donkey anti-mouse IgG	1:1000	R&D Systems Cat #NL007
	NorthernLights™ Streptavidin NL557	1:10.000	R&D Systems Cat #NL999
Nuclear stain	ProLong™ Diamond Antifade Mountant with DAPI		Invitrogen™ Cat # P36962
Primers			
	Target	Forward/ Reverse primers (5′-3′)	
	SOX2	F: ATGTCCCAGCACTACCAGAG/ R: GCACCCCTCCCATTTCCC	
	POU5F1 (OCT3/4)	F: CCTCACTTCACTGCACTGTA/ R: CAGGTTTTCTTTCCCTAGCT	
	NANOG	F: CCAAAGGCAAACAACCCACTT/ R: CGGGACCTTGTCTTCCTTTTT	
	PBGD	F: GGAGCCATGTCTGGTAACGG/ R: CCACGCGAATCACTCTCATCT	

for 1 h at RT (Table 2). ProLong™ Diamond Antifade Mountant with DAPI was used for nuclear counterstaining. Imaging was performed using confocal microscopy (Nikon Eclipse TE300).

3.5. Karyotype analysis

Genomic DNA from iPSCs at passage 10–15 was isolated using QIAamp® DNA Mini Kit (catalog no. #51304) and analysed with the hiPSC Genetic Analysis Kit (Catalog # 07550, Stemcell Technologies) according to the manufacturer's guidelines and analysed with "*STEMCELL Technologies Genetic Analysis Tool*" (https://shiny.stemcell.com/ShinyApps/psc_genetic_analysis_app/).

3.6. Mutation analysis

To confirm pathogenic mutations in the *ST3GAL5* gene, we performed sequencing analysis by Next Generation Sequencing (NGS), using AmpliSeq for Illumina Custom DNA panel, running on MiniSeq (Illumina) platform and Sanger Sequencing Method.

3.7. Analysis of differentiation potential

Differentiation potential was assessed by direct differentiation of iPSCs in all three germ layers, using STEMdiff™ Trilineage Differentiation Kit (Catalog # 05230, Stem Cell Technologies). Markers of ectoderm, mesoderm and endoderm were analysed by immunocytochemistry to confirm the differentiated cells' identity.

3.8. STR analysis

We performed STR analysis of genomic DNA from parental fibroblasts and iPSCs using AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit (Applied Biosystems™): 15 STR loci, including CODIS 13 plus D2S1338 and D19S433, and amelogenin.

CRediT authorship contribution statement

Rodolfo Tonin: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Federica Feo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation. **Silvia Falliano:** Methodology. **Laura Giunti:** Methodology. **Martino Calamai:** Methodology. **Elena Procopio:** Writing – review & editing, Resources. **Francesco Mari:** Data curation. **Vittorio Sciruicchio:** Investigation. **Valerio Conti:** Writing – review & editing. **Ilaria Fanelli:** Methodology. **Franco Bambi:** Supervision. **Renzo Guerrini:** Writing – review & editing, Supervision. **Amelia Morrone:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103431.

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