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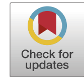
Lab Resource: Single Cell Line



Generation of a cellular model for mucopolysaccharidosis type IVA (MPS IVA) (AOUMEYi003-A) from a patient carrying compound heterozygous mutations p.G116V and p.G290S in the *GALNS* gene

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A B S T R A C T

Mucopolysaccharidosis type IVA (MPS IVA) is an autosomal recessive lysosomal storage disorder (LSD) caused by a deficiency of enzyme N-acetylgalactosamine-6-sulfatase (GALNS), characterised by systemic skeletal dysplasia and joint abnormalities with respiratory, cardiac and visceral manifestations. We generated a human induced pluripotent stem cell (hiPSC) line derived from MPS IVA patient's fibroblasts. The patient was compound heterozygous for the known p.(Gly116Val) and p.(Gly290Ser) in the *GALNS* gene. We used a reprogramming RNA-based method. This hiPSC line was positive for "Yamanaka" factors and able to differentiate into all three germ layers, confirming its pluripotency potential.

Resource Table:

Unique stem cell line identifier	AOUMEYi003-A
Alternative name(s) of stem cell lines	N/A
Institution	Meyer Children's Hospital IRCCS, Department of Neuroscience and Medical Genetics, Florence, Italy
Contact information of distributor	Amelia Morrone – amelia.morrone@meyer.it Rodolfo Tonin – rodolfo.tonin@meyer.it
Type of cell lines	iPSC
Origin	Human
Additional origin info required	AOUMEYi003-A Age: 2 years Sex: Male Ethnicity: Central European
Cell Source	Skin fibroblasts
Clonality	Mixed
Method of reprogramming	RNA-based vector
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Mucopolysaccharidosis IVA (MPS IVA)
Gene/locus	*GALNS*; NM_000512.5(GRCh38): 16:88,813,734–88,856,947
Date archived/stock date	N/A
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/AOUMEYi003-A
Ethical approval	The Pediatric Ethics Committee of Tuscany Region (Italy) approved the study (No 58/2024). Written informed consent was obtained from the patients' legal guardians.

1. Resource utility

The generation of AOUMEYi003-A line offers a promising tool to carry out research on the pathophysiology of MPS IVA. Such studies are challenging due the limited amount of available appropriate cell lines or patient-specific material (Leal et al., 2023). The hiPSC-MPS IVA model may be a valuable resource for drug screening.

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2. Resource details

Morquio A syndrome is a rare disorder caused by bi-allelic pathogenetic variants in the N-acetylgalactosamine 6-sulfatase encoding gene (*GALNS*). The GALNS enzyme is responsible for the lysosomal degradation of glycosaminoglycans (GAGs), chondroitin 6-sulfate (C6S) and keratan sulfate (KS), under physiological conditions. Patients with impaired GALNS activity suffer skeletal deformities, low stature, hearing loss, ocular clouding and cardiac and pulmonary dysfunctions caused by accumulation of C6S and KS in multiple tissues (Khan et al., 2017). Clinical heterogeneity has been described. A hiPSC-based MPS IVA model is a promising tool as it has the potential to differentiate into disease-relevant cell types such as chondrocytes, macrophages, myocytes, osteoblasts and osteoclasts, allowing investigation into specific clinical aspects of the syndrome.

In this study, we established a human iPSC line from skin fibroblasts of a 3-year old boy carrying compound heterozygous mutations of a c.347G > T p.(Gly116Val) variant in exon 4 (Morrone et al., 2014) and a c.868G > A p.(Gly290Ser) variant in exon 8 (Tomatsu et al., 1997) of the *GALNS* gene. The patient had a clinical diagnosis of Morquio syndrome, with absent enzyme activity and keratan sulfate in urine. Splenomegaly due to hereditary spherocytosis.

We reprogrammed skin fibroblasts using a non-integrating RNA-based method (OKSGM-vector). The resulting hiPSC line named AOUMEYi003-A exhibited a classical embryonic stem cell morphology (bright-field image in Fig. 1 A), a normal male karyotype (46, XY) lacking chromosomal abnormalities as confirmed by cytogenetic analysis (Fig. 1 C) and expressed the canonical stemness markers NANOG, SOX2, OCT3/4 and KLF4 and, as assessed by both immunofluorescence staining (Fig. 1 A) and RT-qPCR analysis (Fig. 1 B).

We used Next Generation Sequencing (NGS) to confirm disease-causing mutations in the *GALNS* gene in the established hiPSC line (Table 1, Fig. 1 E). This hiPSC line was not contaminated with mycoplasma (Supplementary Data 2). Genotype identity was verified by short tandem repeat (STR) DNA analysis which showed matching genotypes at all 16 loci examined in AOUMEYi003-A line and in the starting fibroblast cell line (Fig. 1 F and Supplementary Data 1).

We performed directed differentiation protocol *in vitro* to assess the pluripotency potential of the AOUMEYi003-A line. The expression analysis of specific differentiation markers confirmed the ability of the established hiPSC-MPS IVA line to differentiate into cells of all three germ layers (Ectoderm, Mesoderm and Endoderm) (Fig. 1 D). AOUMEYi003-A line was validated for clearance of residual self-replicating RNA vector and resulted vector-free.

3. Materials and methods

3.1. Skin biopsy and isolation of patient's fibroblasts

In order to obtain a primary culture of fibroblasts, we used a dermal puncher for skin biopsy from the patient's forearm. We cultured the biopsy sample for two weeks using Dulbecco's modified Eagle's medium (DMEM-Ref: 41,965 – 035 Gibco) supplemented with fetal bovine serum (10 %), 2 mM of L-glutamine, and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). Informed consent was obtained. The sample was anonymised and used for research purposes only.

3.2. iPSC reprogramming and cell culture

We reprogrammed human skin patient's fibroblasts at passage 3 using ReproRNA™-OKSGM Kit (Catalog #05930, Stemcell Technologies) according to the manufacturer's guidelines. 17–18 days after transfection we picked up manually iPSC colonies. 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. We maintained cells in a humidified atmosphere at 37 °C with 5 % CO2.

3.3. RT-qPCR

We isolated total RNA from iPSC clones at passage 10 and from fibroblasts with RNeasy Mini Kit according to the manufacturer's guidelines (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

We fixed cells grown on Matrigel-coated coverslips at passages 10–15 with 4 % paraformaldehyde (PFA) for 10 min at 37 °C, we permeabilized them with 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. The cells were then incubated overnight at 4 °C with the primary antibodies listed in Table 2. These antibodies were detected by secondary antibody staining for 1 h at RT (Table 2). ProLong™ Diamond Antifade Mountant with DAPI (cat no.#P36962) was used for nuclear counterstaining. Imaging was performed by confocal microscopy (Nikon Eclipse TE300).

3.5. Karyotype analysis

We isolated genomic DNA from iPSCs at passage 10–15 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 *GALNS* 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. Total DNA from iPSC clones was isolated using QIAamp® DNA Mini Kit (catalog no. #51304).

3.7. Analysis of differentiation potential

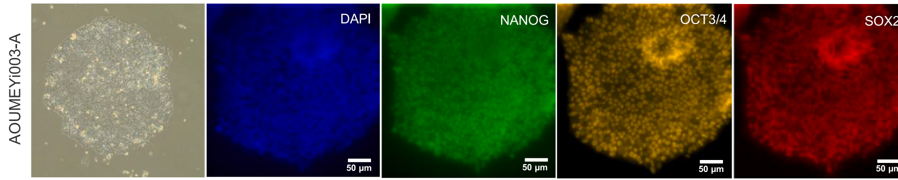
We assessed differentiation potential by direct differentiation of iPSCs at passage 15 in all three germ layers using the STEMdiff™ Trilineage Differentiation Kit (Catalog # 05230, Stem Cell Technologies). Markers of ectoderm, mesoderm and endoderm were analysed by immunofluorescence to confirm the identity of the differentiated cells.

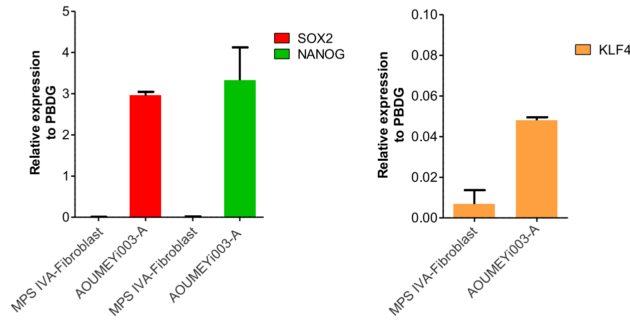
3.8. STR analysis

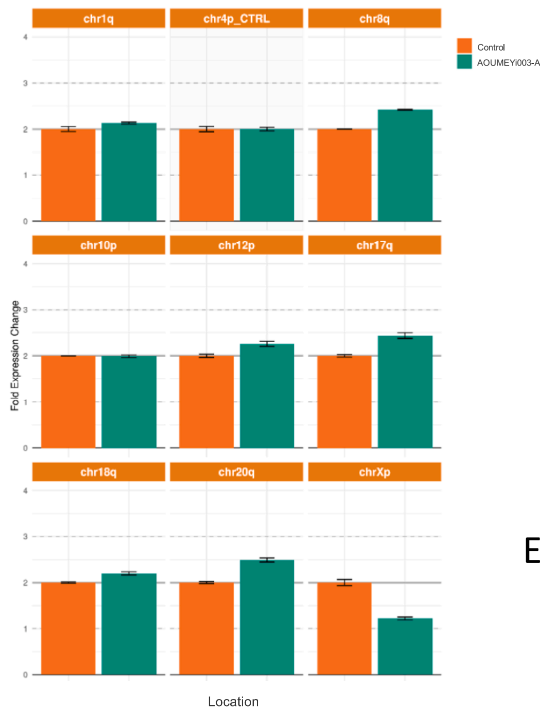
We performed STR analysis of genomic DNA from parental fibroblasts and iPSCs using AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit (Applied Biosystems™): 16 STR loci, including CODIS 13 plus D2S1338 and D19S433, and amelogenin.

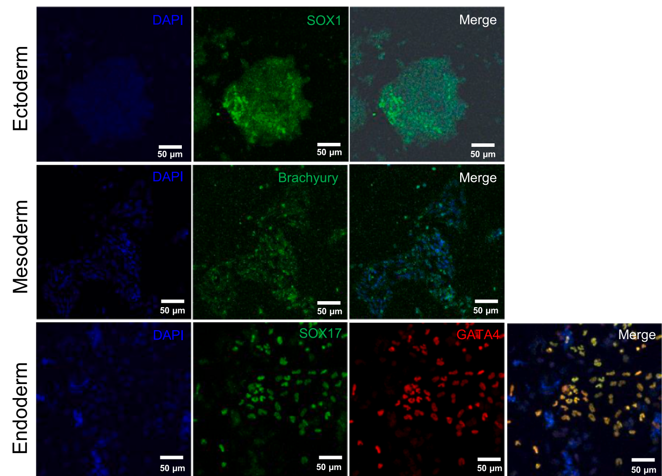
3.9. Mycoplasma detection

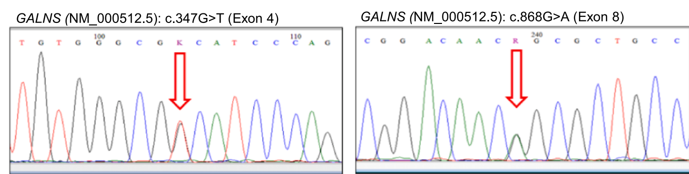
We performed analysis of mycoplasma contamination by a PCR-











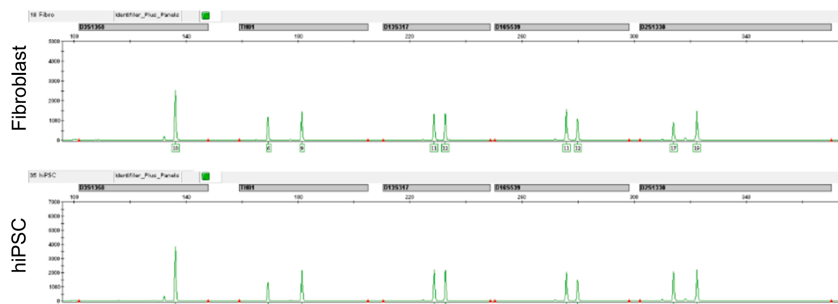


Fig. 1. Characterization of AOUMEYi003-A iPSC line

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis byImmunocytochemistry	hiPCS line expresses pluripotency markers: NANOG, OCT4 and SOX2	Fig. 1 panel A
	Quantitative analysis byRT-qPCR	hiPCS line expresses pluripotency markers: *SOX2, NANOG* and *KLF4*	Fig. 1 Panel B
Genotype	Karyotype	AOUMEYi003-A: 46, XYhiPCS 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 F; Supplementary Data 1
Mutation analysis	Sanger Sequencing	hiPSC line is compound heterozygous for pathogenic variants in *GALNS* gene	Fig. 1 Panel E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma detection by PCR	Negative	Supplementary Data 2 *(*Siegl et al., 2023*)*
Differentiation potential	Directed differentiation	Expression of germlayer-specific markers: Ectoderm (SOX1), Mesoderm (BRACHYURY), and Endoderm (SOX17, GATA4)	Fig. 1 panel D

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′)**	**Product Lenght**	
SOX2	F: ATGTCCCAGCACTACCAGAG/R: GCACCCCTCCCATTTCCC	141 bp	
KLF4	F: GGTCGGACCACCTCGCCTTACA/R: CTCAGTTGGGAACTTGACCA	172 bp	
NANOG	F: CCAAAGGCAAACAACCCACTT/R: CGGGACCTTGTCTTCCTTTTT	62 bp	
PBGD	F: GGAGCCATGTCTGGTAACGG/R: CCACGCGAATCACTCTCATCT	76 bp	
Targeted mutation analysis (GALNS, exon 4)	F: ACGCAGCCCCCAGTGTCC/R: GACGCCTGGGCAGGCGT	366 bp	
Targeted mutation analysis (GALNS, exon 8)	F: TCCAGGTATGGAGATGCCTT/R: TCCAGGCACTCTTCGCTGA	500 bp	

based method exploiting the specificity of a pair of primers designed by Siegl et al., and specific for highly conserved regions of mycoplasma 16S rRNA. The analysis was performed on total DNA extracted from iPSC colonies and the fibroblast line using the EZ1® DSP Virus Kit (ref. 62724). The specific primers are listed in Table 2. The PCR product (191 bp) was detected on 2 % agarose gel.

CRediT authorship contribution statement

Federica Feo: Data curation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. **Silvia Falliano:** Methodology, Resources, Validation. **Anna Caciotti:** Resources, Validation. **Marina Rinaldi:** Methodology, Validation. **Alessia Caroli:** Methodology. **Laura Giunti:** Data curation, Methodology. **Martino Calamai:** Data curation, Methodology. **Elena Procopio:** Conceptualization, Investigation, Writing – review & editing. **Renzo Guerrini:** Conceptualization, Supervision, Writing – review & editing. **Amelia Morrone:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision. **Rodolfo Tonin:** Conceptualization, Data curation, Investigation, Supervision, Writing – original draft, 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.

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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.2025.103746.

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