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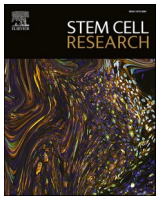

Lab Resource: Genetically-Modified Single Cell Line

Generation of human induced pluripotent stem cell line UGENTi001-A from a patient with Marfan syndrome carrying a heterozygous c.7754 T > C variant in *FBN1* and the isogenic control UGENT001-A-1 using CRISPR/Cas9 editing

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A B S T R A C T

Marfan syndrome is an autosomal dominant genetic disorder resulting from pathogenic variants in *FBN1* gene. *FBN1* encodes for fibrillin-1, an important extracellular matrix protein. Impaired fibrillin-1 affects multiple organ systems, including the cardiovascular system. We generated an iPSC line carrying a heterozygous variant c.7754 T > C (p.Ile2585Thr, missense) in *FBN1* from a patient with Marfan syndrome. Also, an isogenic control is generated, where the pathogenic variant is repaired using CRISPR-Cas9. This isogenic pair provides a valuable resource for *in vitro* disease modelling.

Resource Table

Unique stem cell line identifier	UGENTi001-A and UGENTi001-A-1
Alternative name(s) of stem cell line	UGENT-MFS003 and UGENT-MFS003-CRISPR
Institution	Ghent University
Contact information of the reported cell line distributor	Jolanda van Hengel; Jolanda.vanHengel@UGent.be
Type of cell line	iPSC
Origin	Human
Additional origin info *(applicable for human ESC or iPSC)*	Age: 34 Sex: Male Ethnicity if known: Caucasian
Cell Source	Renal epithelial cells from urine
Method of reprogramming	Integration-free Sendai virus expressing human OCT3/4, SOX2, KLF4, c-MYC
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-qPCR
Cell culture system used	Feeder-free, Geltrex coating, Essential 8 medium

(*continued on next column*)

Resource Table (*continued*)

Unique stem cell line identifier	UGENTi001-A and UGENTi001-A-1
Type of Genetic Modification	Gene correction
Associated disease	Marfan syndrome (OMIM:154700)
Gene/locus	Gene: *FBN1* (MIM:134797) Location: 15q21.1 Pathogenic variant: c.7754 T > C (p.Ile2585Thr, missense), heterozygous
Method of modification/site-specific nuclease used	CRISPR/Cas9
Site-specific nuclease (SSN) delivery method	RNP
All genetic material introduced into the cells	ssODN
Analysis of the nuclease-targeted allele status	Sanger sequencing PCR product
Method of the off-target nuclease activity surveillance	N/A
Name of transgene	N/A
Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific)	N/A

(*continued on next page*)

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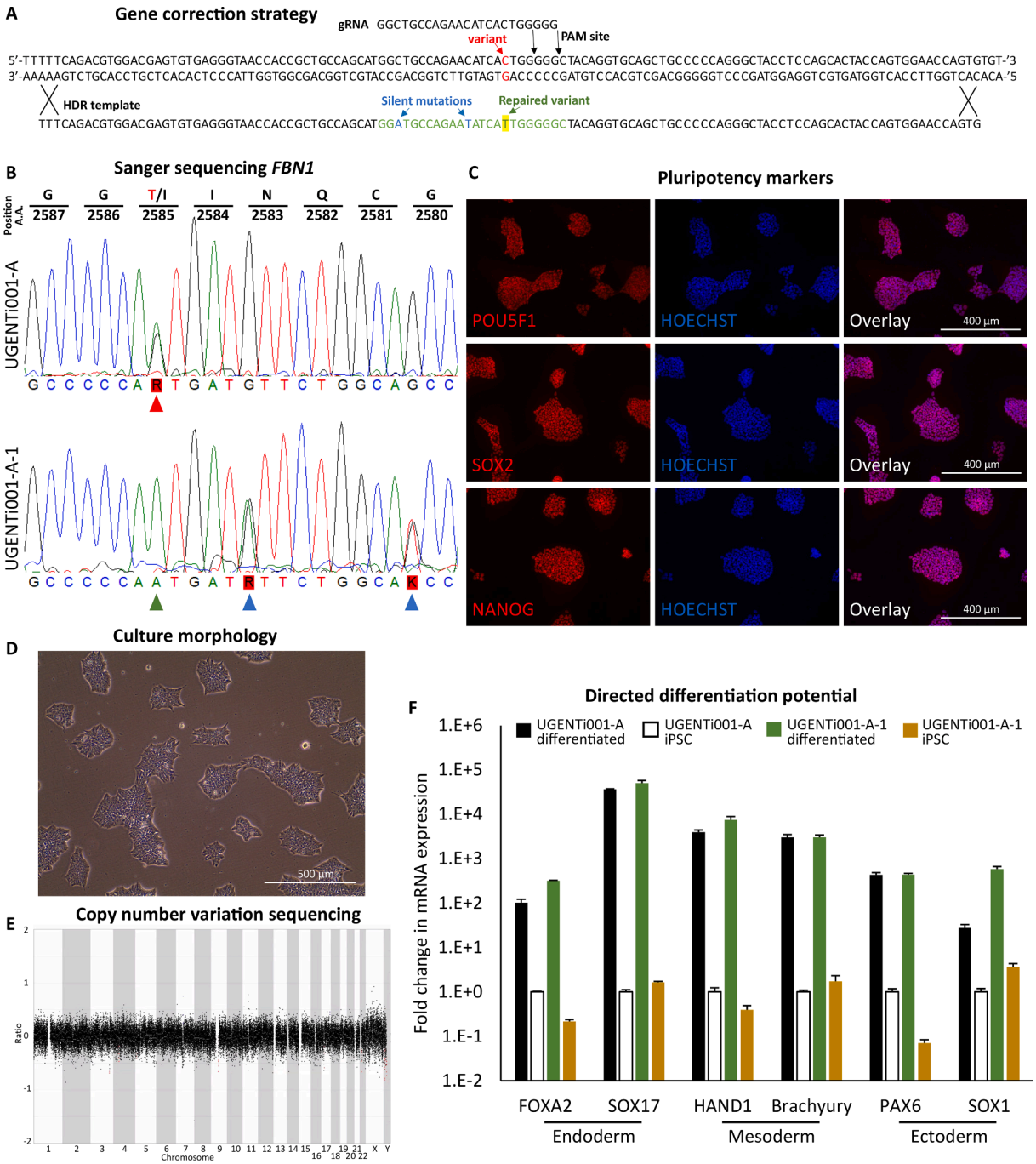

Figure 1. Characteristics of iPSC line UGENTi001-A-1.

Resource Table (*continued*)

Unique stem cell line identifier	UGENTi001-A and UGENTi001-A-1
Inducible/constitutive system details	N/A
Date archived/stock date	10/2022
Cell line repository/bank	hPSCreg: https://hpscreg.eu/cell-line/UGENTi001-A and https://hpscreg.eu/cell-line/UGENTi001-A-1
Ethical/GMO work approvals	Ethics Committee of University Hospital Ghent: EC2019/1511
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	N/A

1. Resource utility

The generated iPSC line UGENTi001-A carrying a heterozygous variant c.7754 T > C (p.Ile2585Thr, missense) in *FBN1* from a patient with Marfan syndrome and the isogenic control UGENTi001-A-1 can be differentiated into smooth muscle cells, cardiomyocytes and other cell types to study the role of the pathogenic variant on aortopathy and Marfan-related cardiomyopathy.

2. Resource details

Marfan syndrome is an autosomal dominant multisystem disorder that is characterized by manifestations in the cardiovascular, ocular, skeletal, integumentary, and respiratory organ systems (Milewicz et al.,

Table 1
Characterization and validation.

Classification (optional *italicized*)	Test	Result	Data
Morphology	Phase-contrast microscopy	Normal	Fig. 1D
Pluripotency status evidence for the described cell line	Qualitative analysis *(Immunocytochemistry)*	Positive for POU5F1, SOX2 and NANOG	Fig. 1C
	Quantitative analysis *(RT-qPCR)*	Not performed	N/A
Karyotype	CNV-seq	46 XY (normal)	Fig. 1E
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	Heterozygous template repair as evidenced by presence of silent mutations	Fig. 1B
	Transgene-specific PCR	Not performed	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	Not performed	N/A
Parental and modified cell line genetic identity evidence	STR analysis	16 loci tested, all matched	Submitted in the archive with journal
		D3S1358, TH01, D21S11, D18S51, PENTA E, D5S818, D13S317, D7S820 D16S539, CSF1PO, PENTA D, AMEL, vWA, D8S1179, TPOX, FGA	
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR)	Heterozygous template repair as evidenced by presence of silent mutations	Fig. 1B
	PCR-based analyses	Not performed	N/A
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	Not performed	N/A
Off-target nuclease analysis-	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	Not performed	N/A
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by RT-PCR, negative	N/A
Multilineage differentiation potential	Directed differentiation	Positive for *FOXA2* and *SOX17* (endoderm), *HAND1* and *Brachyury* (mesoderm), *PAX6* and *SOX1* (ectoderm)	Fig. 1F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype - additional histocompatibility info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

2021). The most frequently seen complications involve aortic root dilation, leading to potential aneurysm, dissection, and rupture. Aortopathy is the leading cause of mortality and morbidity in patients with Marfan syndrome, however, in a subset of patients primary myocardial dysfunction and sudden death are observed, independently from aortic- or valvular disease. Little is known about the mechanisms leading to primary Marfan-related cardiomyopathy.

Marfan syndrome is caused by pathogenic variants in the *FBN1* gene that codes for the extracellular matrix protein fibrillin-1 which is one of the main components of microfibrils, that provide structure and support to the extracellular matrix. Besides structural functions, fibrillin-1 has important mechanosignalling functions. Pathogenic variants in *FBN1* result in a structurally and functionally impaired matrix that causes compensatory effects such as excessive collagen and proteoglycan deposition, in turn leading to progressive weakening and stiffness of the extracellular matrix. To date, more than 3000 different pathogenic variants in *FBN1* have been described with a limited genotype-phenotype correlation.

iPSC technology is a promising tool to decipher the mechanisms responsible for Marfan-related cardiomyopathy and is complimentary to existing *in vivo* mice models. Previously, we reported on the first *in vitro* model for Marfan-related cardiomyopathy (Aalders et al., 2020) using cardiomyocytes differentiated from an iPSC line carrying a pathogenic variant in exon 30 of *FBN1* (c.3725G > A). Functional characterisation of this cardiac model revealed several abnormalities in the behavior of Marfan cardiomyocytes.

Here we describe a renal epithelial cell-derived iPSC line carrying a heterozygous variant c.7754 T > C in *FBN1*. Sendai virus enabled the delivery of the Yamanaka factors Oct3/4, Sox2, c-Myc and Klf4. To obtain monoclonal colonies, the transduced iPSCs were manually picked for at least five expansions. Clearance of Sendai viral vectors was confirmed by RT-qPCR for SeV and c-Myc. The generated iPSC line UGENTi001-A harboring the pathogenic variant was then corrected using CRISPR/Cas9 technology (Fig. 1A). Sanger sequencing confirmed the homology directed repair (green arrow in Fig. 1B) and was further evidenced by the presence of the two silent mutations (blue arrows in Fig. 1A, B). STR-analysis revealed a full match between UGENTi001-A, UGENTi001-A-1 and the renal epithelial cells from the donor for all 16 loci (Table 1).

Bright field microscopy shows normal morphology of the UGENTi001-A-1 (Fig. 1D). Pluripotency was confirmed by immunohistochemical stainings for POU5F1, SOX2 and NANOG (Fig. 1C). CNV-seq did not reveal any chromosomal abnormalities in the generated iPSC lines (passage 16, Fig. 1E). Mycoplasma contamination was absent as assessed by RT-qPCR analysis. RT-qPCR analysis of directed differentiations towards endoderm (evidenced by *FOXA2* and *SOX17*), mesoderm (*HAND1* and *Brachyury*) and ectoderm (*PAX6* and *SOX1*) confirmed the differentiation potential of UGENTi001-A-1 (Fig. 1F).

In conclusion, we have successfully established an isogenic iPSC pair for a heterozygous variant c.7754 T > C in *FBN1*. These iPSCs lines are a valuable source to further advance and complement the existing *in vitro* models for Marfan-related cardiomyopathy and aortopathy in Marfan.

3. Materials and methods

3.1. Ethics

All the experimental protocols were approved by the Ethics Committee of University Hospital Ghent: (EC2019/1511).

3.2. Reprogramming of renal epithelial cells

At least 50 ml urine was collected and processed as previously described (Zhou et al., 2012). Cells were passaged at a confluency of 80 % using TrypLE Select 1X (Life Technologies). Approximately 60,000 renal epithelial cells were seeded in a gelatin coated 6-well (6250 cells/cm^2). Transfection using CytoTune-iPS 2.0 Sendai Reprogramming Kit (Life Technologies) was initiated at a confluency of 30 %, following manufacturer's protocol. Colonies were manually picked for at least five passages. Cultures that were enzymatically passaged using TrypLE were supplemented with Revitacell (Life Technologies) in Essential 8 medium

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT3/4 (POU5F1)	1:600	Santa Cruz Cat# SC-5279 RRID: AB_628051
	Rabbit anti-SOX2	1:800	Life Technologies Cat# PA1-094, RRID: AB_2539862
	Rabbit anti-NANOG	1:600	Life Technologies Cat# PA1-097, RRID: AB_2539867
Secondary antibodies	Goat anti-Mouse IgG Dylight 594	1:500	Life Technologies Cat# 35511 RRID: AB_1965950
	Goat anti-Rabbit IgG Dylight 594	1:500	Life Technologies Cat# 35561 RRID: AB_1965951
Nuclear stain	Hoechst 33342	1:1000	Life Technologies Cat# H3570
Site-specific nuclease			
Nuclease information	Alt-R® S.p. HiFi Cas9 Nuclease V3	IDT Cat# 1081060	
Delivery method	Nucleofection with 4D-Nucleofector	Lonza	
Selection/enrichment strategy	N/A	N/A	

Primers and Oligonucleotides used in this study

	Target	**Forward/Reverse primer (5′-3′)**
Sendai viral vector	SeV	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
	c-Myc	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
Differentiation Markers (RT-qPCR)	*FOXA2* (endoderm)	TACAGGCGCAGCTACACGCACGCAAAG/GCGGGGCACCTTCAGGAAACAGTCGT
	SOX17 (endoderm)	GTGGACCGCACGGAATTTG/GGAGATTCACACCGGAGTCA
	HAND1 (mesoderm)	TGCCTGAGAAAGAGAACCAG/ATGGCAGGATGAACAAACAC
	Brachyury (mesoderm)	GGATGAAGGCTCCCGTCTC/GCTGTGATCTCCTCGTTCTGATA
	PAX6 (ectoderm)	GTGTCCAACGGATGTGTGAG/CTAGCCAGGTTGCGAAGAAC
	SOX1 (ectoderm)	CCTGTGTGTACCCTGGAGTTTCTGT/TGCACGAAGCACCTGCAATAAGATG
Reference Genes (RT-qPCR)	*B2M*	TGCTGTCTCCATGTTTGATGTATCT/TCTCTGCTCCCCACCTCTAAGT
	RPL13A	CCTGGAGGAGAAGAGGAAAGAGA/TTGAGGACCTCTGTGTATTTGTCAA
Genotyping	FBN1-39 (c.7754 T > C)	CAATAACACATTTACAGCTTCAGA/CTTAGCAGCAGTTCCAGAAG
Targeted mutation sequencing	FBN1-39 (c.7754 T > C)	Fig. 1B
gRNA	FBN1-39 (c.7754 T > C)	GGCTGCCAGAACATCACTGGGGG
SSODN		TTTCAGACGTGGACGAGTGTGAGGGTAACCACCGCTGCCAGCATGGATGCCAGAATATCATTGGGGGCTACAGGTGCAGCTGCCCCCAGGGCTACCTCCAGCACTACCAGTGGAACCAGTG
Mycoplasma detection (RT-qPCR)	Mycoplasma	ACACCATGGGAGCTGGTAAT/CCTCATCGACTTTCAGACCCAAGGCAT

(Life Technologies) on Geltrex (Life Technologies) coated plates at 37 °C, 5 % O_2, 5 % CO_2. Medium was renewed daily and cells were passaged single-cell in ratio 1:6-1:10 every 3-4 days using TrypLE at 80 % confluency. The absence of Sendai viral vectors was confirmed by RT-qPCR, RNA was extracted using GenElute Total RNA Purification Kit (Sigma), cDNA was transcribed by cDNA Synthesis Kit (Biotech Rabbit) and RT-qPCR was performed using Sybergreen PCR mix (Life Technologies), primers listed in Table 2.

3.3. Generation of isogenic line

The generated iPSC line UGENTi001-A carrying a heterozygous variant c.7754 T > C was corrected using CRISPR/Cas9 induced homology directed repair. A specific gRNA (IDT) was designed using Benchling (Table 2). Two silent mutations were incorporated in the ssODN (IDT). Delivery of the RNP complex and ssODN was achieved through nucleofection using a 4D-Nucleofector (Lonza) according to manufacturer's instructions (program DN100, P3 primary cell solution). After nucleofection, cells were seeded in Essential 8 medium supplemented with Alt-R HDR Enhancer (IDT) and Revitacell on a geltrex coated 6-well. By using a dilution series, clones were screened for the desired base correction by Sanger sequencing, primers listed in Table 2.

3.4. Sanger sequencing

Genomic DNA was extracted using QuickExtract DNA Extraction Solution (Lucigen-epicentre). The DNA sequence of interest was amplified using PCR with Kappa2G Robust (Roche). Repair of the heterozygous variant c.7754 T > C was verified using Sanger sequencing. Primers used are listed in Table 2.

3.5. CNV-seq

Molecular karyotyping was performed by sWGS on a Hiseq3000 sequencer (Illumina) with a minimal resolution of 100 kb.

3.6. STR analysis

Genomic DNA of UGENTi001-A, UGENTi001-A-1 and the source renal epithelial cells was analyzed and compared using Powerplex 16 system (Promega) according to manufacturer's instructions.

3.7. Directed differentiation

Differentiation of iPSCs was directed to the three germ layers using the STEMdiff Trilineage Differentiation Kit (Stem Cell Technologies) according to manufacturer's instructions. RNA was isolated at day 5 (ectoderm and mesoderm) or 7 (endoderm) and RT-qPCR was performed, primers are listed in Table 2.

3.8. Immunohistochemistry

iPSCs were fixed in 4 % paraformaldehyde for 20 min and incubated with primary antibodies overnight at 4 °C, listed in Table 2, and then incubated with secondary antibodies overnight at 4 °C. Nuclei were stained with HOECHST. Images were made with with EVOS FL cell imaging system (Thermo Fisher).

3.9. Mycoplasma testing

Absence of Mycoplasma contamination in iPSCs was confirmed by RT-qPCR. Primers listed in Table 2.

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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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