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Generation of two iPSC lines from vascular Ehlers-Danlos Syndrome (vEDS) patients carrying a missense mutation in COL3A1 gene

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Abstract

Vascular Ehlers-Danlos Syndrome (vEDS) is an inherited connective tissue disorder caused by *COL3A1* gene, mutations that encodes type III collagen, a crucial component of blood vessels. vEDS can be life-threatening as these patients can have severe internal bleeding due to arterial rupture. Here, we generated induced pluripotent stem cell (iPSC) lines from two vEDS patients carrying a missense mutation in the *COL3A1* (c.226A > G, p. Asn76Asp) gene. These lines exhibited typical iPSC characteristics including morphology, expression of pluripotency markers, and could differentiate to all three germ layer. These iPSC lines can serve as valuable tools for elucidating the pathophysiology underlying vEDS.

Keywords

vascular Ehlers-Danlos Syndrome; Induced pluripotent stem cells; COL3A1

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CRediT authorship contribution statement

Amit Manhas: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dipti Tripathi:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Formal analysis, Data curation. **Chikage Noishiki:** Methodology. **David Wu:** Methodology. **Lu Liu:** Methodology. **Karim Sallam:** Validation, Investigation. **Jason T. Lee:** Project administration, Funding acquisition. **Eri Fukaya:** Resources. **Nazish Sayed:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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.

1. Resource utility

Patients carrying a missense variation in the *COL3A1* gene (c.226A > G, p.Asn76Asp) showed vascular Ehlers-Danlos Syndrome (vEDS) disease phenotype. iPSCs generated from these patients would serve as a valuable tool to model vEDS disease in a dish for prospective drug screening.

2. Resource details

Ehlers-Danlos Syndrome (EDS) is a group of inherited disorders that primarily affect the connective tissues in the body such as the skin, joints (ligaments, cartilage), and blood vessels. Patient with EDS usually exhibit joint flexibility and stretchy, fragile skin. Among EDS, vascular Ehlers-Danlos Syndrome (vEDS) is the most severe form of the disorder that is characterized by a defect in the synthesis of type III collagen, a critical component of the blood vessel walls that maintains the structural integrity of arteries and internal organs. These defects are due to mutations in the *COL3A1* gene that encodes the pro-alpha1 chains of type III collagen. As a result, there is disruption in the assembly of type III collagen fibrils leading to weakened blood vessels and tissues that are prone to rupture. Indeed, patients with a single nucleotide missense variation in the *COL3A1* gene have exhibited severe vascular complications such as hypertension and varicose veins and carry an increased risk of arterial rupture that can lead to hemorrhage and subcutaneous bleeding. Currently, there are no specific medications that can correct the underlying collagen defect in vEDS, however, for patients with symptomatic or high-risk arterial aneurysms, vascular grafting is considered a necessary surgical intervention to repair the weakend blood vessels (Frank et al., 2019; Frank et al., 2015).

By utilizing patient-specific induced pluripotent stem cells (iPSCs), we can establish a robust *in vitro* screening platform to model the vEDS phenotype in a dish. This approach involves generating iPSCs from patients carrying the missense mutations in the *COL3A1* (c.226A > G, p. Asn76Asp) gene associated with vEDS. These iPSCs can then be differentiated into endothelial cells (iPSC-ECs) and vascular smooth muscle cells (iPSC-VSMCs) that can recapitulate the cellular components of blood vessels affected by vEDS and model the associated disease phenotype in-a-dish. Moreover, this iPSC platform can enable us to conduct high-throughput drug screening assays, which can provide insights into potential therapeutic interventions for vEDS-associated vascular complications (Alqahtani et al., 2022).

Here, we generated iPSC lines from two female patients, a 49-year-old (BFVSBi003-A) and a 53-year-old (BFVSBi004-A), both carrying the *COL3A1* (c.226A > G, p.Asn76Asp) gene missense mutation (Table 1). Patient's peripheral blood mononuclear cells (PBMCs) were reprogrammed into iPSCs using a Sendai virus vector containing Oct3/4, Sox2, Klf4, and c-Myc (Yamanaka factors). These iPSC clones exhibited typical iPSC morphology (Fig. 1A) and expressed pluripotency markers OCT3/4, NANOG, and SOX2, as demonstrated by immunostaining (Fig. 1B). Subsequent differentiation assays confirmed the ability of these iPSC lines to differentiate into endoderm, mesoderm, and ectoderm lineages (Fig. 1C). Furthermore, reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

analysis revealed expression of NANOG and SOX2 at the mRNA level in both lines (Fig. 1D). Importantly, the iPSC lines were free of the Sendai virus vector (Fig. 1E). The presence of the *COL3A1* gene (c.226A > G, p.Asn76Asp) single nucleotide missense variation was confirmed by Sanger sequencing (Fig. 1G). Karyotype analysis using KaryoStat assays demonstrated normal karyotypes for both lines (Fig. 1H), and mycoplasma contamination was absent (Fig. 1F). Lastly, short tandem repeat (STR) analysis confirmed the genetic origin of these iPSC lines, matching that of their respective donor PBMCs **(submitted in archive with journal).**

3. Materials and methods

3.1. Isolation, culture, and reprogramming of PBMCs to iPSCs

PBMCs were isolated from whole blood using Percoll density gradient medium (GE Healthcare, #17089109) as previously described (Tripathi et al., 2024). The isolated PBMCs were purified with Dulbecco's Phosphate Buffered Saline (DPBS) and plated in 24-well plate. The culture medium consisted of StemPro®-34 Serum-Free Medium (SFM) (ThermoFisher Scientific, #10639011) supplemented with StemPro®-34 Nutrient Supplement (ThermoFisher Scientific). Specific growth factors and cytokines, including Stem Cell Factor (SCF) (Peprotech, 100 ng/mL), FLT3 ligand (FLT3) (ThermoFisher Scientific, 100 ng/mL), Interleukin-3 (IL-3) (Peprotech, 20 ng/mL), Interleukin-6 (IL-6) (ThermoFisher Scientific, 20 ng/mL), and Erythropoietin (EPO) (ThermoFisher Scientific, 20 ng/mL), were added to the culture medium to support cell proliferation. Briefly, 2.5×10^5 PBMCs were plated and reprogrammed using the CytoTune-iPSC 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific, #A16517). Transduced PBMCs were plated onto Matrigel-coated plates and cultured in StemPro™-34 medium (Thermo Fisher Scientific). After seven days, the medium was switched to StemMACS™ iPS-Brew medium (Miltenyi Biotec, #130 104-368), and cells were maintained for an additional ten to fifteen days. Colonies were picked, and clones were expanded as previously described (Sayed et al., 2020).

3.2. Maintenance of Induced Pluripotent Stem Cells (iPSCs)

iPSCs were cultured in StemMACS™ iPS-Brew XF medium supplemented as specified (#130-104-368, Miltenyi Biotec) at 37 °C in a humidified atmosphere until they were 95 % confluent. The cells were detached using 0.5 mM EDTA, resuspended in a ROCK inhibitor medium (Selleck Chemicals, #Y27632), and replated onto Matrigel-coated plates. The medium was refreshed after 24 h and every other day until the cells reached confluence.

3.3. Trilineage differentiation assay

To assess the pluripotency of iPSCs, cells were differentiated into endoderm, mesoderm, and ectoderm using a trilineage differentiation kit (STEMCELL Technologies, #05110).

3.4. Immunofluorescence staining

iPSCs or iPSC-differentiated germ layers were fixed with 4 % paraformaldehyde, permeabilized with digitonin, and blocked with Bovine Serum Albumin (BSA) and serum (Donkey Serum or Goat Serum). After overnight incubation with primary antibodies (Table

2), cells were incubated with respective secondary antibodies. Nuclei were counter-stained with NucBlue (ThermoFisher Scientific, #R37606) before imaging.

3.5. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from iPSCs using TRIzol® and Direct-zol™ RNA Miniprep Kit (Zymo Research, #R2052) at passage 14. cDNA was synthesized using the iScript™ cDNA Synthesis Kit (BioRad, #1708891), followed by RT-PCR analysis of NANOG, SOX2, and SEV using TaqMan™ Gene Expression Assay (Applied Biosystems, #4444556).

3.6. Karyotyping

At passage 12, iPSCs were collected and analyzed for chromosomal abnormalities using the KaryoStat™ assay (ThermoFisher).

3.7. Short Tandem Repeat (STR) analysis

Genomic DNA was isolated from PBMCs and iPSCs using the DNeasy Blood & Tissue Kit (Qiagen, #56304) at passage 15. DNA amplification was performed using the CLA IdentiFiler™ Direct PCR Amplification Kit (Thermo Fisher, #A44660), and the products were analyzed by capillary electrophoresis.

3.8. Sequencing

Genomic DNA extracted from iPSC lines was subjected to an amplification with the help of NEB High-Fidelity PCR kit (New England Biolabs, #M0541S) using customized primers (Table 2) at passage 13. The PCR products were sequenced using the ABI3130xl platform (Stanford PAN facility) after purification with the QIAquick Purification Kit (Qiagen, #28706).

3.9. Mycoplasma detection

Mycoplasma contamination was assessed using the MycoAlert Detection Kit (Lonza, #LT07-705).

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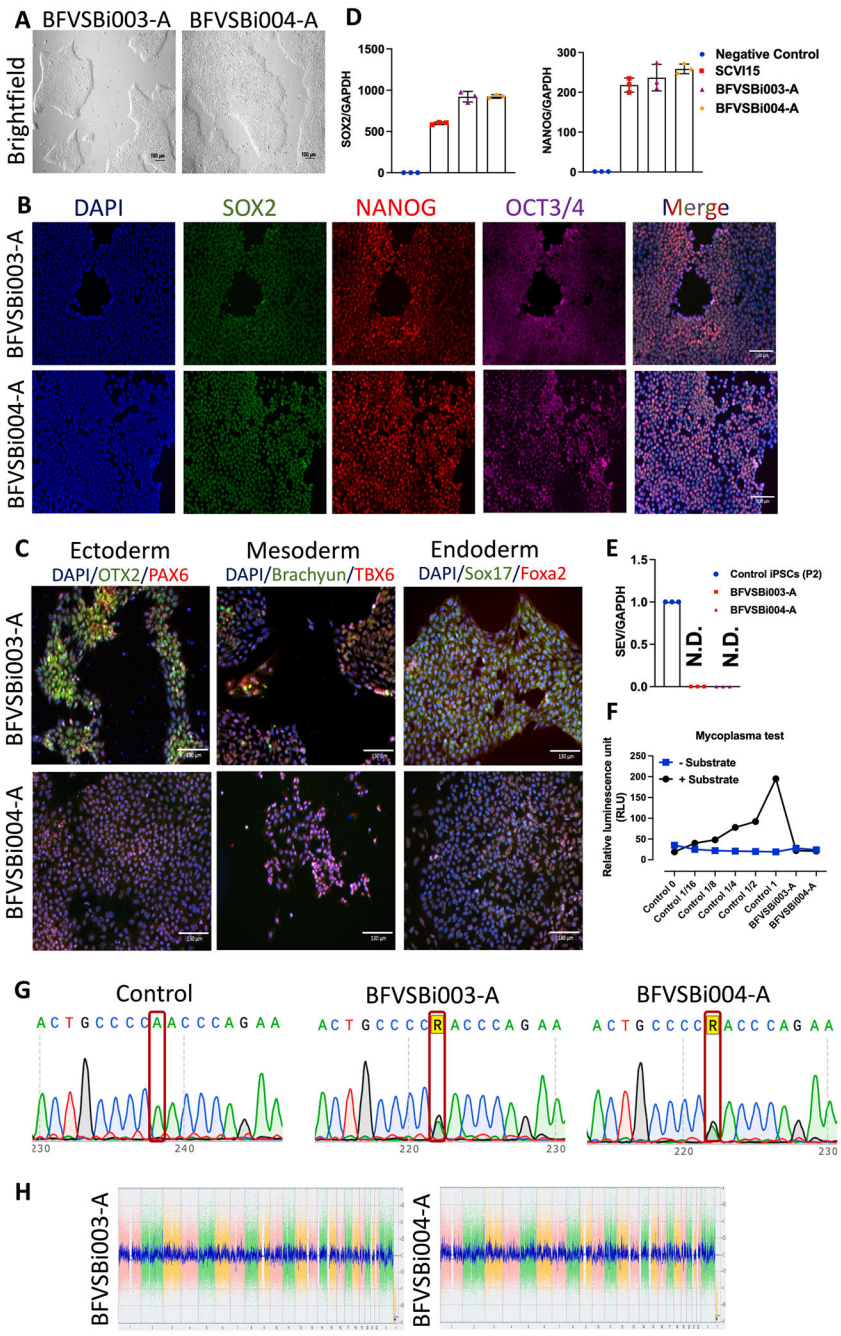

Fig. 1.

Table 1

Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive expression of pluripotency markers: Oct3/4, NANOG, SOX2	Fig. 1B
	Quantitative analysis (RT-qPCR)	mRNA expression of SOX2 and NANOG	Fig. 1D
Genotype	Karyotype: Whole genome array (KaryoStat™ Assay) Resolution 1–2 Mb	Normal karyotype: 46 XX for BFVSBi003-A XX for BFVSBi004-A	Fig. 1H
Identity	Microsatellite PCR (mPCR) or STR analysis	N/A	N/A
		16 loci tested, 100 % matching indentity	Submitted in archive with journal
Mutation analysis	Sequencing	COL3A1 gene (c.226A > G, p.Asn76Asp)	Fig. 1G
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Luminescence: Negative	Fig. 1F
Differentiation potential	Directed differentiation, Immunofluorescence staining for 2 markers per germ layer	Positive Immunofluorescence staining of three germ layer markers **Ectoderm:** PAX6, OTX2 **Endoderm:** SOX17, FOXA2, **Mesoderm:** BRACHYURY, TBX6	Fig. 1C
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	Rabbit Anti-NANOG	1:200	Proteintech Cat# 142951-1-AP,	AB_1607719
	Mouse IgG2bκ Anti-OCT-3/4	1:200	Santa Cruz Biotechnology Cat# sc-5279	AB_628051
	Mouse IgG1κ Anti-SOX2	1:200	Santa Cruz Biotechnology Cat# sc-365823	AB_10842165
Ectoderm Markers	Goat Anti-OTX2	1:200	R&D Systems Cat# 963273	AB_2157172
	Rabbit Anti-Pax6	1:100	Thermo Fisher Scientific Cat# 42-6600	AB_2533534
Endoderm Markers	Goat Anti-SOX17	1:200	R&D Systems Cat# 963121	AB_355060
	Rabbit Anti-Foxa2	1:250	Thermo Fischer Scientific Cat# 701698	AB_2576439
Mesoderm Markers	Goat Anti-Brachyury	1:200	R&D Systems Cat# 963427	AB_2200235
	Rabbit Anti-Tbx6	1:200	Thermo Fischer Scientific cat # PA5-35102	AB_2552412
Secondary Antibodies	Alexa Fluor 488 Goat Anti-Mouse IgG1	1:1000	Thermo Fisher Scientific #A-21121	AB_2535764
	Alexa Fluor 488 Donkey Anti-Goat IgG (H + L)	1:1000	Thermo Fisher Scientific #A-11055	AB_2534102
	Alexa Fluor 555 Goat Anti-Rabbit IgG (H + L)	1:500	Thermo Fisher Scientific #A-21428	AB_141784
	Alexa Fluor 647 Goat Anti-Mouse IgG2b	1:250	Thermo Fisher Scientific #A-21242	AB_2535811
Primers				
	Target	**Forward/Reverse primer (5′-3′)**		
Sendai Virus	Sendai Virus genome	Mr04269880_mr		
Genotyping	COL3A1 gene (c.226A > G, p.Asn76Asp)	Fwd: TTTCAAACCTTTTCAACTTTGGC Rev: CTTACTGGATCTCCCTTGGGG		
House-Keeping Gene	GAPDH	HS02758991_g1		
	SOX2	HS01053049_s1		
	NANOG	HS02387400_g1		

Resource Table

Unique stem cell lines identifier	1. BFVSBi003-A 2. BFVSBi004-A
Alternative name(s) of stem cell lines	1. VS-18 2. VS-19
Institution	Baszucki Family Vascular Surgery Biobank
Contact information of distributor	Dr. Nazish Sayed sayedns@stanford.edu
Type of cell lines	iPSC
Origin	Human
Additional origin info (Applicable for human ESC or iPSC)	1. BFVSBi003-A; Age:49, Sex: Female; Ethnicity: Caucasian 2. BFVSBi004-A; Age:53, Sex: Female; Ethnicity: Caucasian
Cell Source	PBMCs
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus expressing human OCT4, SOX2, KLF4, and c-MYC
Genetic Modification	Yes
Type of Genetic Modification	Spontaneous mutation
Evidence of the reprogramming transgene loss	RT/q-PCR
Associated disease	vascular Ehlers-Danlos Syndrome (vEDS)
Gene/locus	1. COL3A1 (c.226A > G, p.Asn76Asp)
Date archived/stock date	1. BFVSBi003-A: 12-17-2023 2. BFVSBi004-A: 11-23-2023
Cell line repository/bank	1. https://hpscreg.eu/cell-line/BFVSBi003-A 2. https://hpscreg.eu/cell-line/BFVSBi004-A
Ethical approvals	The Administrative Panel approved the generation of the lines on Human Subjects Research (IRB) under IRB #62122, “Human Induced Pluripotent Stem Cells for Studying Cardiac and Vascular Diseases.”