Stem Cell Research 31 (2018) 216–221

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Multiple cell lines

Generation of three spinocerebellar ataxia type-12 patients derived induced pluripotent stem cell lines (IGIBi002-A, IGIBi003-A and IGIBi004-A)

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A B S T R A C T

Spinocerebellar ataxia type-12 (SCA12) is a neurological disorder caused due to triplet (CAG) repeat expansion in 5' UTR of *PPP2R2B*. It is one of the most prominent SCA-subtype in Indian population and till date no patient specific models have been described. Human-induced-pluripotent-stem cell (HiPSC) based disease modelling has become the next generation tool for studying various human pathologies. In the present study we established three SCA12 patient specific iPSC lines. All the generated lines have shown pluripotency markers, normal karyotype, in-vitro three germ layers differentiation potential, vector clearance, SCA12 mutation, parental genomic identity and contamination free culture.

Resource table

Unique stem cell lines identifier	1) IGIBi002-A 2) IGIBi003-A 3) IGIBi004-A
Alternative names of stem cell lines	1) SCA12_001 2) SCA12_002 3) SCA12_003
Institution	Institute of Genomics and Integrative Biology (CSIR-IGIB), Delhi
Contact information of distributor	Dr. Mohammed Faruq, MBBS, PhD Genomics and Molecular Medicine, CSIR- Institute of Genomics and Integrative Biology, Mall Road, Delhi 110,007 Email: faruq.mohd@igib.res.in
Type of cell lines	Induced Pluripotent Stem Cell lines (iPSCs)
Origin	Human
Cell Source	Peripheral blood mononuclear cells (PBMCs) derived Lymphoblastoid cell lines (LCLs)
Clonality	Mixed
Method of reprogramming	Episomal Plasmids containing OCT3/4-shP53-F, SOX2, KLF4, L-MYC and LIN28
Multiline rationale	Three iPSC lines of varying CAG repeat length
Gene modification	No
Type of modification	N/A
Associated disease	Spinocerebellar Ataxia Type-12 (SCA12)
Gene/locus	*PPP2R2B*/5q 32
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	December 2016
Cell line repository/ bank	N/A
Ethical approval	The study was approved by Institute Ethics Committee & Institutional Committee for Stem Cell Research, All India Institute of Medical Sciences, New Delhi (Ref no. IEC/NP-26/2013 & IC-SCRT/19/14R) and the Human Ethics committee of Institute of Genomics and Integrative Biology, Delhi (GENCODE; BSC0123).

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https://doi.org/10.1016/j.scr.2018.08.008
Received 19 June 2018; Received in revised form 18 July 2018; Accepted 8 August 2018
Available online 14 August 2018

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
IGIBi002-A	SCA12_001	Male	49 Years	Indian	*PPP2R2B* has 14/59 CAG repeats	SCA12
IGIBi003-A	SCA12_002	Female	50 Years	Indian	*PPP2R2B* has 10/67 CAG repeats	SCA12
IGIBi004-A	SCA12_003	Male	49 Years	Indian	*PPP2R2B* has 17/65 CAG repeats	SCA12

1. Resource utility

These generated iPSC lines are useful in disease modelling and therapeutic intervention. These generated lines can be used to study the mechanistic role of CAG repeat expansion in SCA12 pathogenesis and prospective translational research could be harnessed by these cell types in-vitro to identify and screen drugs useful for SCA12 therapeutics.

2. Resource details

SCA12 is an autosomal dominant trinucleotide repeat expansion disorder clinically characterized by cerebro-cerebellar degeneration (i.e. progressive action tremor of hands, gait ataxia, dysarthria, head tremor etc.) and variable onset of other pyramidal and extrapyramidal features during the course of disease progression (Holmes et al., 1999). The underlying genetic defect in SCA12 is the expansion of CAG repeats ($\geq$43) in 5′ region of *PPP2R2B* (Srivastava et al., 2017).

In the present study peripheral blood samples of clinically and genetically SCA12 patients (n = 3) (Table 1) were collected at All India Institute of Medical Sciences (AIIMS), New Delhi. Information about the onset and course of the disease, initial symptoms, medical history and medication was obtained with a semi-structured interview.

To generate induced pluripotent stem cells (iPSCs), peripheral blood mononuclear cells (PBMCs), were isolated and were transformed to lymphoblastoid cell lines (LCLs) using Epstein Barr virus (EBV) following a published protocol (Frisan et al., 2001). LCLs were characterized for the presence of B cell subpopulation and SCA12 mutation. Immunophenotying revealed 87–88% to be positive for B cells in culture and genotyping identified retention of SCA12 mutation in diseased state (Supplemental Fig. 1 panel A–C). LCLs have expanded population doubling and thus serve as a continuous resource of donor cells avoiding repeated sampling. The LCLs were tested for sample integrity (STR analysis with the original donor sample) and sterility (mycoplasma detection) prior to reprogramming. In this study we report successful generation of three iPSC lines IGIBi002-A (SCA12_001), IGIBi003-A (SCA12_002) and IGIBi004-A (SCA12_003) from unrelated symptomatic SCA12 patients having varying CAG repeats length. Reprogramming was performed using electroporation of episomal plasmids expressing reprogramming factors OCT4, SOX2, L-MYC, KLF4, LIN-28 and P53 shRNA as described previously (Okita et al., 2011). As early as 12–15 days after electroporation, typical human embryonic stem (ES) cells like colonies were observed. All the lines showed typical iPSCs- like morphology and retinyl esters sequestered blue fluorescence (Fig. 1 panel A and B) when cultured in standard HuES medium containing 20% KOSR, characteristic of 'primed state' as reported previously (Muthusamy et al., 2014). Established iPSCs were characterized with respect to their pluripotent properties and were positive for pluripotency markers including OCT4, SOX2, SSEA4 (Fig. 1 panel B, E and Supplemental Fig. 3 panel A). Generated iPSC lines were validated to ensure chromosomal stability and had shown normal karyotype (Fig. 1 panel C, Table 2). Genotyping of iPSCs identified *PPP2R2B*-CAG expansion (mutation causing SCA12) (Fig. 1 panel F) (fragment analysis data not shown). All these iPSC lines exhibited potential to differentiate into three cellular lineages (ectoderm, mesoderm and endoderm) as evident from PluriTest (Supplemental Fig. 2 panel A–C) and their ability to form embryoid bodies which expressed NESTIN, OTX2 (ectodermal), nodal, SNAI1 (mesodermal) and GATA4, AFP (endodermal) transcripts in-vitro, followed by immunostaining of three cellular lineages (Fig. 1 panel D, G, H and Supplemental Fig. 3 panel B, Table 2). The short tandem repeat (STR) profiling of these lines were identical to PBMCs and LCLs from which the respective lines were derived, indicated that these lines are derivative of respective donor without cellular cross-contamination (Table 2). The exogenous reprogramming vector loss was confirmed by not detection of plasmids foot print after several passages (Fig. 1 panel I). Further all these lines were tested for mycoplasma contamination and were found sterile. To our knowledge, this is the first study to generate SCA12 specific iPSCs and their transcriptomic data resources for future studies.

3. Materials and methods

3.1. Subjects and study approval

Patients were clinically and genetically confirmed for the presence of SCA12 at All India Institute of Medical Sciences (AIIMS) before their recruitment into this study. For iPSC generation, blood samples of three unrelated patients were drawn. The study was approved by the Institute Ethics Committee & Institutional Committee for Stem Cell Research, AIIMS, New Delhi (Ref no. IEC/NP-26/2013 & IC-SCRT/19/14R) and the Human Ethics committee of CSIR-IGIB for GENCODE (BSC0123). Informed written consent was obtained from each subject.

3.2. Peripheral blood mononuclear cells (PBMCs) isolation, LCL generation and characterization

PBMCs were isolated from three unrelated symptomatic SCA12 patients using Histopaque (Sigma-Aldrich, cat # 10771) gradient following manufacturer's instructions. PBMCs were then transformed to lymphoblastoid cell lines (LCLs) using Epstein Barr virus (EBV) following a published protocol (Frisan et al., 2001). LCLs were maintained in RPMI 1640 medium (Gibco, cat # 61870-010) supplemented with 20% FBS (Gibco cat # 10082147), and 1× Penicillin & Streptomycin (Invitrogen, cat #15140-122). Direct immunofluorescence was measured to confirm the presence of the B cell subpopulation using CD19 cell surface marker. CD3 staining was also performed to confirm the exclusion of T cells in culture. Cells were washed once with 1 X DPBS (Gibco, cat # 59321C), suspended in FACS buffer containing diluted FcR blocking and incubated on ice 10 min. Blocking was removed and cells were resuspended in FACS buffer and antibodies labelled with fluorophore (CD19- PerCP-Cy5.5 and CD3-APC, BD Biosciences, cat# 555335, & 561,295) as per manufactures instructions. Cells were then incubated on ice for 30 min, washed once with FACS buffer, resuspended in PBS and acquired on FACS Aria (BD Biosciences).

3.3. Induced pluripotent stem cells (iPSCs) generation

The generated LCLs of the three patients were reprogrammed via episomal plasmids harboring reprogramming factors as described previously (Okita et al., 2011). Briefly 0.2 million LCLs were electroporated with 1 μg of three reprogramming plasmids viz., pCXLE-hOCT3/4-shP53-F (Addgene, cat #27077), pCXLE-hSK (Addgene, cat #27078) and pCXLE-hUL (Addgene, cat #27080) using Neon device (Invitrogen, USA). Post electroporation, cells were transferred onto

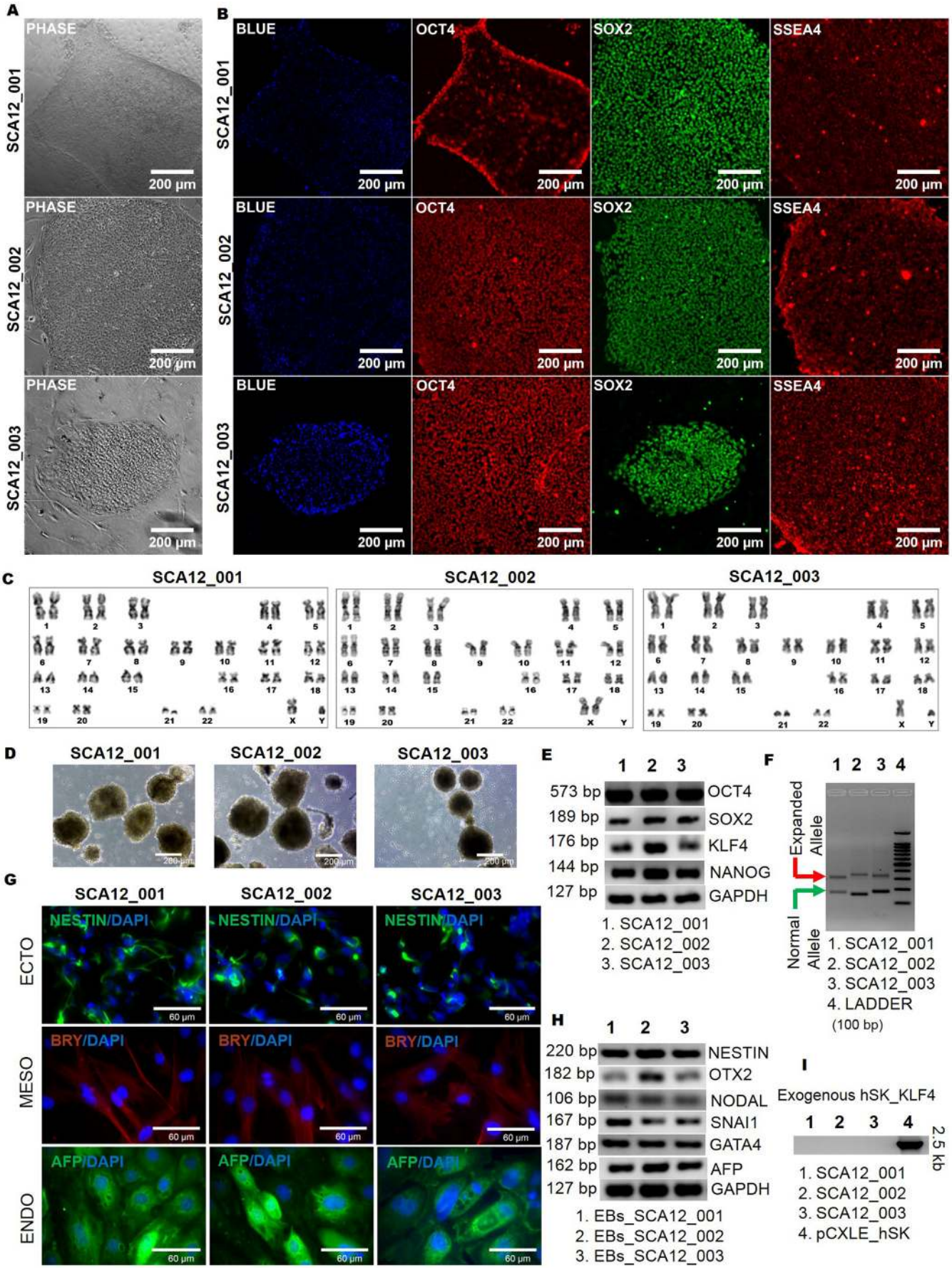

Fig. 1. Generation and characterization of iPSC lines (IGIBi002-A, IGIBi003-A and IGIBi004-A)

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal (scale bar: 200 μm)	Fig. 1 panel A
Phenotype	Qualitative analysis (RT-PCR)	Expression of transcripts of pluripotency markers including *OCT4, SOX2, KLF4, NANOG*	Fig. 1 panel E and supplemental Fig. 3 panel A
	Qualitative analysis (Immunocytochemistry)	Immunopositivity of pluripotency markers OCT4, SOX2 and SSEA4 (scale bar: 200 μm)	Fig. 1 panel B
	In-silico	PluriTest (All the lines have passed the PluriTest score)	Supplemental Fig. 2 panel A-C
Genotype	Karyotype (G-banding)	SCA12_001: 46XY (band resolution: 250), SCA12_002: 46XX (band resolution: 300), SCA12_003:46XY (band resolution: 250)	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR)	Not performed	N/A
	STR analysis	10 Loci tested, Matched	Submitted in archive with journal
Mutation analysis	CAG Repeat length analysis by PCR and fluorescence based fragment analysis	Genotyping of iPSCs identified *PPP2R2B*-CAG expansion mutation	Fig. 1 panel F and data (fragment analysis) not shown but available from author
	Southern Blot OR WGS	Not done	N/A
Microbiology and virology	Mycoplasma	By luminescence - Negative	Data not shown but available from author
Differentiation potential	Embryoid body formation (RT-PCR)	Ectoderm: *NESTIN,OTX2,* Mesoderm: *NODAL, SNAI1* Endoderm:*GATA4, AFP*	Fig. 1 panel H
	Embryoid body formation (Immunocytochemistry)	EBs: Phase contrast (scale bar: 200 μm) Ectoderm: NESTIN Mesoderm: Brachyury/Bry Endoderm: AFP (scale bar: 60 μm)	Fig. 1 panel D Fig. 1 panel G
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not tested	N/A
Genotype additional info	Blood group genotyping	Not done	N/A
	HLA tissue typing	Not done	N/A

gamma irradiated mouse embryonic fibroblast feeder layer and cultured in iPSC medium containing KnockOut DMEM (Gibco, cat #10829-018), 20% KnockOut Serum Replacement (Gibco, cat #10828-028), 55 mM beta-mercaptoethanol (Gibco, cat #21985-023), 10 mM nonessential amino acids (Gibco, cat #11140-050), 2 mM L-glutamine (Gibco, cat #35050-061), 1× Penicillin & Streptomycin (Invitrogen, cat #15140-122), 10 ng/ml recombinant human bFGF (Gibco, cat #PHG6015) supplemented with 50 μg/ml L-ascorbic acid (Sigma-Aldrich, cat # A4403), and 0.5 mM sodium butyrate (Sigma-Aldrich, cat # B5887). Reagents were obtained from Life Technologies except stated otherwise. Once the colonies grew, they were mechanically passaged onto inactivated mouse embryonic fibroblasts (MEF) feeder layers and propagated in iPSC medium lacking sodium butyrate and ascorbic acid.

3.4. Lipid body associated retinyl esters fluorescence (blue fluorescence)

Blue fluorescence is an endogenous marker associated with pluripotency (Muthusamy et al., 2014). 'Primed' pluripotent stem cells exhibit a characteristic blue fluorescence arising from retinyl esters sequestered in cytoplasmic lipid bodies. This endogenous fluorescence correlates with the expression of standard pluripotency markers like OCT4, NANOG etc., iPSC lines cultured in standard HuES medium containing KnockOut DMEM (Gibco, cat #10829-018), 20% KnockOut Serum Replacement (Gibco, cat #10828-028), 55 mM beta-mercaptoethanol (Gibco, cat #21985-023), 10 mM nonessential amino acids (Gibco, cat #11140-050), 2 mM L-glutamine (Gibco, cat #35050-061), 1× Penicillin & Streptomycin (Invitrogen, cat #15140-122), 10 ng/ml recombinant human bFGF (Gibco, cat #PHG6015) were allowed to achieve 70–80% confluency. The blue fluorescence was visualized by epifluorescence microscopy using DAPI filter (excitation/emission); (325–375 nm/450–500 nm). The images were acquired using Nikon Eclipse TE2000-E-PFS, Japan.

3.5. Immunostaining

The iPSCs were fixed with 4% paraformaldehyde (PFA) for 20 min followed by permeabilization with 0.1% Triton- X 100 for 10 min. The cells were kept in blocking solution (1% BSA) for one hour at room temperature, incubated overnight at 4 °C with diluted primary antibodies (Table 3). The cells were washed thrice with 1× HBSS (Gibco, cat # 59321C) (5 min per wash) and incubated for 1 h with diluted secondary antibodies at room temperature. Counter staining for nuclei was done with DAPI (Thermo Fisher Scientific, cat # 62248) for 5 min at room temperature. Slides were mounted in DABCO and imaging was done using epifluorescence microscope (Nikon Eclipse TE2000-E-PFS, Japan).

3.6. Karyotyping

All the generated lines were karyotyped using G banding techniques. The iPSCs were cultured 40 min in colchicine (100 μg/ml, Gibco #15212-012) to arrest the chromosomes at the metaphase stage of the cell cycle, followed by 0.56% KCl treatment. Cells were fixed in Carnoy's fixative (Methanol: Acetic Acid:: 3:1) and cell spreads were made by dropping of cell suspension onto the slides for G-Banding analysis.

3.7. CAG repeat analysis for detection of expansion mutation

Genomic DNA was extracted from cells using QIAamp DNA Mini Kit (Qiagen, cat # 51304). PCR assay was used to amplify CAG repeats region of *PPP2R2B* using primer sequences (Table 3). Fragment analysis was performed on Applied Biosystems 3730xl Genetic Analyzer and the length of CAG repeats was calculated using GeneMapper software (Applied Biosystems).

3.8. Microsatellite short tandem repeat analysis

Short tandem repeat analysis was done using GenePrint 10 System (Promega # B9510). The isolated DNA was used to amplify for 10 STR loci (TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818, D21S11, and amelogenin) following manufacturer's instructions. Fragment analysis was performed on Applied Biosystems 3730xl Genetic Analyzer, and allelic sizes were calculated using GeneMapper software (Applied Biosystems).

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Rabbit Anti-OCT4	1:100	Thermo Fisher Scientific, Cat# A24867, RRID: AB_2650999
Pluripotency markers	Rat Anti-SOX2	1:100	Thermo Fisher Scientific, Cat# A24759, RRID: AB_2651000
Pluripotency markers	Mouse Anti-SSEA4	1:100	Thermo Fisher Scientific Cat# A24866, RRID: AB_2651001
Differentiation Markers	Mouse Anti-Nestin	1:200	Abcam, Cat# ab22035, RRID: AB_446723
Differentiation Markers	Rabbit Anti-Brachyury/Bry	1:200	Abcam, Cat# ab20680, RRID: AB_727024
Differentiation Markers	Mouse Anti- Alpha Fetoprotein (AFP)	1:200	Santa Cruz Biotechnology, Cat# sc-130,302, RRID: AB_2223934
Secondary antibodies	Alexa Fluor 488 Goat Anti-Mouse, IgG (H + L) Cross-Adsorbed Secondary Antibody	1:250	Thermo Fisher Scientific,Cat# A24877, RRID: AB_2651008
Secondary antibodies	Alexa Fluor 488 Donkey Anti-Rat, IgG (H + L) Highly Cross-Adsorbed Secondary Antibody	1:250	Thermo Fisher Scientific,Cat# A-24876, RRID: AB_2651007
Secondary antibodies	Alexa Fluor 594 Goat Anti-Mouse, IgG (H + L) Cross-Adsorbed Secondary Antibody	1:250	Thermo Fisher Scientific,Cat# A21236, RRID: AB_2535805
Secondary antibodies	Alexa Fluor 488 Donkey Anti-Mouse, IgG (H + L) Highly Cross-Adsorbed Secondary Antibody	1:1000	Molecular Probe, Cat# A-21202, RRID: AB_141607
Secondary antibodies	Alexa Fluor 594 Goat Anti-Rabbit, IgG (H + L) Highly Cross-Adsorbed Secondary Antibody	1:1000	Thermo Fisher Scientific Cat# A-11037, RRID: AB_2534095
FACS	APC Mouse Anti-CD3	N/A	BD Biosciences, Cat# 555335, RRID: AB_398591
	PerCP-Cy5.5 Mouse Anti- CD19	N/A	BD Biosciences Cat# 561295, RRID: AB_10644017

Primers		
Primer name	Target	Forward/reverse primers (5′ - 3′)
Pluripotency markers (RT-PCR)	OCT4	CGACCATCTGCCGCTTTGAG/CCCCCTGTCCCCCATTCCTA
Pluripotency markers (RT-PCR)	SOX2	AGTCTCCAAGCGACGAAAAA/TTTCACGTTTGCAACTGTCC
Pluripotency markers (RT-PCR)	NANOG	ACATGCAACCTGAAGACGTGTG/CATGGAAACCAGAACACGTGG
Pluripotency markers (RT-PCR)	KLF4	AGTTCCCATCTCAAGGCACA/GACCTGGAAAATGCTCGGTC
Pluripotency markers (RT-PCR)	TDGF1	CGATGCTAACGCCTCTTTTC/GGCCAAATGCTGTCATCTCT
Pluripotency markers (RT-PCR)	TERT	TGTGCACCAACATCTACAAG/GCGTTCTTGGCTTTCAGGAT
Pluripotency markers (RT-PCR)	GDF3	CGGGAATGTACTTCGCTTTC/CCCTTTCTTTGATGGCAGAC
Pluripotency markers (RT-PCR)	DNMT3A	GGGGACGTCCGCAGCGTCACAC/CAGGGTTGGACTCGAGAAATCGC
Pluripotency markers (RT-PCR)	REX1	GCGTACGCAAATTAAAGTCCAGA/CAGCATCCTAAACAGCTCGCAGAAT
Pluripotency markers (RT-PCR)	FOXD3	ACTCTGCCTCTCCCCAATTT/TCGGTTTTCGGTTTTACCTG
Differentiation markers (RT-PCR)	NESTIN	AACAGCGACGGAGGTCTCTA/TTCTCTTGTCCCGCAGACTT
Differentiation markers (RT-PCR)	OTX2	AGGTGGCACTGAAAATCAACTT/TTGGCCACTTGTTCCACTCT
Differentiation markers (RT-PCR)	PAX6	AGACACAGCCCTCACAAACA/CATAACTCCGCCCATTCACC
Differentiation markers (RT-PCR)	NODAL	AGACATCATCCGCAGCCTAC/CCATGCCAGATCCTCTTGTT
Differentiation markers (RT-PCR)	DES	CTGCTCAACGTGAAGATGGC/TGGACCTCAGAACCCCTTTG
Differentiation markers (RT-PCR)	SNAI1	ATGTCCGGACCCACACTG/CTTGTGGAGCAGGGACATTC
Differentiation markers (RT-PCR)	AFP	AGGGAGCGGCTGACATTATT/CAGAGAATGCAGGAGGGACA
Differentiation markers (RT-PCR)	GATA4	TCCAAACCAGAAAACGGAAG/CTGTGCCCGTAGTGAGATGA
House-keeping genes (RT-PCR)	GAPDH	CTGAGCTCATTTCCTGGTATGA/CTTCCTCTTGTGCTCTTGCTG
Targeted mutation analysis (PCR & fragment analysis)	SCA12-mutation (*PPP2R2B*-CAG repeat expansion)	FAM –TGCTGGGAAAGAGTCGTG/GCCAGCGCACTCACCCTC
Episomal plasmids (PCR)	Exo_SOX2_KLF4	TTCTTCTTTTTCCTACAGCTCC/TAAAAATGTCTCTTCATGTGTAAGG

3.9. RNA isolation, cDNA synthesis and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from cells using RNeasy Mini Kit (Qiagen, cat #74104). To remove any genomic DNA contamination from isolated RNA samples, DNase treatment was given using TURBO DNA-free Kit (Ambion, cat # AM1907). cDNA was synthesized from 1 μg total RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat# 4368814). Primers were designed using the online tool Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) (Table 3). Reverse transcriptase PCR (RT-PCR) was performed for the assessment of transcripts associated with pluripotency and germ layers (ectoderm, endoderm and mesoderm) (Primers details is provided in Table 3). The amplified products were checked on 2% agarose gel using UV trans-illuminator documentation system (Bio Rad).

3.10. RNA sequencing (RNA-Seq)

RNA-Seq libraries were prepared with 1 μg RNA using TruSeq RNA sample preparation kit. Cluster generation was carried out on cBot using Illumina SBS kit.v3 protocol. Libraries were sequenced on Illumina HiSeq 2500 (Illumina). Generated bcl files were converted into FASTQ using CASAVA v1.8. Trimming and quality filtering was performed using FASTX tool kit (http://hannonlab.cshl.edu/fastx_toolkit/download.html) and FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/download.html#fastqc). The sequenced reads were further mapped to the reference transcriptome (hg38) using Tophat v2.0.5 allowing a maximum of two mismatches. To quantify gene expression, Fragments Per Kilobase of transcript per Million mapped reads (FPKM) was performed using Cufflinks v2.0.2. FPKM were further used by Cuffdiff v2.0.2, in order to estimate differential expression of genes (DEG) between patients and controls.

3.11. Availability of data and materials

For each respective cell line, the generated RNA-Seq data have been deposited in NCBI with accession number SRA110347, with following sample identifiers, IG0002iPSCSCA12 (IGIBi002-A), IG0003iPSCSCA12

(IGIBi003-A) and IG0004iPSCSCA12 (IGIBi004-A).

4. Test for pluripotency

4.1. PluriTest

The RNA-Seq libraries of all the three generated iPSCs were prepared with 1 μg RNA using TruSeq RNA sample preparation kit and sequencing of libraries were carried out on Illumina HiSeq 2500 (Illumina). The generated FASTQ files (*.fastq.gz) were subjected to open source tools PluriTest (https://www.thermofisher.com), to verify pluripotency. PluriTest is a widely accepted method for pluripotency confirmation which compares global gene expression profile of generated lines with whole transcriptome database of human iPSCs. All the lines have passed the PluriTest score.

4.2. In-vitro differentiation

Spontaneous differentiation toward the three lineages was done through embryoid body (EB) formation. For EB generation, manually disintegrated iPSC colonies (clumps containing 200–300 cell) were cultured on non-adherent dish (Corning, cat # 3261) in iPSC medium without basic FGF. Medium was changed alternatively till day 8, followed by total RNA isolation. RT-PCR was performed for the assessment of transcripts associated with different germ layers (ectoderm, endoderm and mesoderm) (Primers details is provided in Table 3). The amplified products were checked on 2% agarose gel using UV trans-illuminator documentation system (Bio Rad).

For Immunofluorescence, generated EBs were plated onto 0.1% gelatine coated dishes and cultured in DMEM/12 (Gibco, cat # 11320-074) with 20% FBS (Gibco cat # 10082147) up to 15 days with media change daily. Further plated cells were immunostained for 3 germ layers markers i.e. Nestin (Ectoderm), Brachyury/Bry (Mesoderm) and AFP (endodermal) as enlisted (Table 3).

4.3. Testing for vector clearance

Genomic DNA of generated iPSC lines was subjected to polymerase chain reaction (PCR). PCR was carried out with primers specific for plasmid DNA (Table 3). The amplified products were checked on 2% agarose gel using UV trans-illuminator documentation system (Bio Rad).

4.4. Mycoplasma detection

Mycoplasma detection was carried out by MycoAlert kit (Lonza, cat # LT07-418) as per manufactures instructions.

Acknowledgements

We acknowledge Varun Suroliya, and Renu Kumari of CSIR-IGIB for assistance in RNA-Seq experiments, Manish Dabas, CSIR-IGIB for STR analysis. We are grateful to all members of Professor Mitradas M. Panicker's laboratory at NCBS for their experimental support. We also acknowledge Adarsh Kr. Mishra, IGIB for assisting in image acquisition, Nishu Tyagi, Bharathram Uppili and Aditi Joshi, IGIB for their analytic support. We thank all the subjects who had participated in this study. This study was supported by Council of Scientific & Industrial Research (CSIR) funded project GENCODE (BSC0123). For OM, funding from Shanta Wadhwani Centre for Cardiac and Neural Research (SWCCNR) and InStem is acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.08.008.

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