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Generation of a human induced pluripotent stem cell line (QBRIi009-A) from a patient with a heterozygous deletion of *FOXA2*

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A B S T R A C T

FOXA2 is a transcription factor, playing an important role during development. We established an induced pluripotent stem cell (iPSC) line, QBRIi009-A, using non-integrating Sendai virus from a 4-year-old boy, displaying a complex clinical phenotype. Molecular karyotyping and cytogenetics confirmed a *de novo* proximal 20p11.2 deletion with a reciprocal translocation between the short arm of chromosome 6 and 20. The deleted region (~969 kb) contains only one gene, *FOXA2*. The generated hiPSC line was fully characterized for its pluripotency and its genetic identity. This iPSC line provides a useful model to study FOXA2 role during human development and in disease pathogenesis.

Resource Table

Unique stem cell line identifier	QBRIi009-A
Alternative name(s) of stem cell line	FOXA2$^{+/-}$ iPSCs
Institution	Qatar Biomedical research institute QBRI, Hamad Bin Khalifa University, Qatar Foundation, Qatar.
Contact information of distributor	Essam M. Abdelalim. E-mail: emohamed@hbku.edu.qa
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 4 years old Sex: Male Ethnicity: Bangali
Cell Source	Blood
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus vector for delivery of OCT3/4, SOX2, c-MYC and KLF4
Genetic Modification	YES
Type of Modification	Hereditary
Associated disease	Multiple syndromic features, growth hormone deficiency and central hypothyroidism
Gene/locus	Gene: FOXA2 Locus: chr20: 22,561,642 - 22,566,101 Deletion: chromosome 20 at bands p11.22 to p11.21
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	Date cell line archived or deposited in repository
Cell line repository/bank	N/A
Ethical approval	Blood samples were obtained from Sidra Medicine hospital with full informed consent. The protocol was approved by the Institutional Review Board (IRB) of Sidra Medicine (no. 1702007608) and QBRI (no. 2018-002)

1. Resource utility

Our iPSC line was generated from a patient with a heterozygous deletion of chromosome 20 at bands p11.22 to p11.21, encompassing only *FOXA2* gene. This iPSC line offers an *in vitro* model to investigate the role of FOXA2 during human development and in the disease development.

2. Resource details

Previous studies reported that 20p11.2 proximal deletions, overlapping *FOXA2* displays multiple phenotypic abnormalities, including developmental delay, panhypopituitarism, heterotaxy and neurodevelopmental abnormalities (Dines et al., 2019; Dayem-Quere et al., 2013). *FOXA2* mutations are associated with hypopituitarism,

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Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel B
Phenotype	Qualitative analysis (Immunocytochemistry and RT-PCR)	Assess staining/expression of pluripotency markers: OCT4, SOX2, NANOG, SSEA4, TRA-1-60, TRA-81, TERT, REX1, DPPA4, cMYC and KLF4.	Fig. 1 panel B and E
	Quantitative analysis (Flow cytometry)	OCT3/4: 98.8%, SOX2: 99.1%, TRA- 1-60: 99.2% and TRA-81: 98%	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46 XY, t (6; 20) (p11; p11) (46, XY, del (20) (p11.21p11.22) with mean resolution of 300.	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	15 loci, 100% matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	G-banding karyotyping WGS	Fig. 1 panel A & C
Microbiology and virology	Mycoplasma Sendai virus	Mycoplasma testing by RT-PCR Negative by RT-PCR	Fig. 1/ Supplementary Fig. 1 panel D
Differentiation potential	Embryoid body formation and Scorecard	The Embryoid body formed and express NESTIN, BRACHYURY SOX17, FOXA2	Fig. 1 panel G, H and I
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

hyperinsulinism, endodermal organ and craniofacial abnormalities (Giri et al., 2017; Vajravelu et al., 2018). Such evidence indicates a key role of *FOXA2* in the regulation of a wide variety of biological processes, including gastrulation, neuron development and endodermal lineage development (Kaestner, 2010). However, further investigations are required to reveal the mechanistic role of *FOXA2,* especially in the human model.

Our iPSC line was generated from a 4-year-old boy with a heterozygous deletion of the short arm of chromosome 20 at bands p11.22 to p11.21, with a reciprocal translocation between short arm of chromosome 6 and 20. The cytogenic microarray analysis showed ~969 kb deletion from 21,881,142 – 22,850,635 that contains only one OMIM gene, *FOXA2*. Investigations confirmed growth hormone (GH) deficiency, central hypothyroidism, in addition to craniofacial dysmorphism, urogenital, and gastrointestinal anomalies.

PBMCs isolated from the patient's blood sample were transduced with non-integrating Sendai virus vectors expressing OCT4, SOX2, c-MYC, and KLF4. Around twenty-five days later, the reprogrammed iPSCs colonies were picked up and expanded for further characterization and validation (Table 1). Our analysis showed that the heterozygous deletion of 20p11.22 to 20p11.21 contains only the *FOXA2* gene (Fig. 1A). The morphology of the QBRIi009-A colonies showed similar characteristics of human embryonic stem cells (hESCs) with well-defined borders and high nuclear to cytoplasmic ratio and stained positive for alkaline phosphatase activity (Fig. 1B). The expression of the pluripotency markers (OCT4, SSEA4, SOX2, TRA-1-60, NANOG, and TRA81) was confirmed in the iPSC colonies using immunofluorescence analysis (Fig. 1B). G-banding analysis showed diploid (46, XY) karyotype of the QBRIi009-A line with translocation between the short arm of chromosome 6 and 20, karyotype: [46 XY, t (6; 20) (p11; p11)] and a deletion (46, XY, del (20) (p11.21p11.22) (Fig. 1C). The silencing of the Sendai viral vector was confirmed using RT-PCR at passage 17 (Fig. 1D). The expression of the pluripotency markers (OCT4, SOX2, NANOG, KLF4, c-MYC, TERT, REX1 and DPPA4) were further confirmed on the mRNA level by RT-PCR (Fig. 1E). Flow cytometry analysis demonstrated that the iPSC line expressed high levels of OCT4, SOX2, TRA-1-60, and TRA-81 (Fig. 1F). Spontaneous differentiation using the embryoid body (EB) technique showed the ability of the iPSC line to differentiate into the three lineages, including ectoderm (NESTIN+), mesoderm (BRACHYURY+), and endoderm (SOX17+) (Fig. 1G). These results were confirmed using the scorecard analysis, which showed high scores for the three germ layers and lost the pluripotency markers (Fig. 1H). Mycoplasma test confirmed that QBRIi009-A was mycoplasma-free (Supplementary Fig. 1). FOXA2 haploinsufficiency was confirmed in the QBRIi009-A-derived definitive endoderm in comparison to control iPSCs using Western blotting (Fig. 1I). Furthermore, STR analysis authenticated the identity of the cell line with the parental PBMCs (Supplementary Table).

3. Materials and methods

3.1. Cell culture and reprogramming

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll Paque (Sigma Aldrich) and cultured in StemPro 34 complete media. Isolated cells were reprogrammed using CytoTune-iPS 2.0 Sendai reprogramming kit (Thermo Fisher Scientific). Selected clones were cultured onto Matrigel-coated plates (Corning) in mTesR-1 media (Stem Cell Technologies), with media being changed every day, at 37 °C and 5% CO_2. When the cells reached 70–80% of confluency, they were incubated in ReLeSR (Stem Cell Technologies, Canada) for 3–5 min and passaged by resuspension in mTesR-1 medium supplemented with 10 μM Y-27632 (Rock inhibitor) (Stemgent, USA).

3.2. Alkaline phosphatase assay

The generated iPSCs were stained using alkaline phosphatase kit SCR004, Merck Millipore, USA) following the manufacturer's instruction.

3.3. Immunocytochemistry and flow cytometry

The cells were fixed using 4% paraformaldehyde in 0.1 M PBS, permeabilized in 0.1 M PBS, 0.5% Triton X-100 (PBST), and blocked in 6% bovine serum albumin (BSA) in PBST. The primary antibodies (Table 2) were added overnight at 4 °C. After washing three times with 0.3% Tween 20 in tris-buffered saline (TBST), the cells were incubated for 1 h at room temperature with the secondary antibodies (Table 2). Inverted fluorescence microscope (Olympus IX 53) was used to acquire the images. For flow cytometry, stained cells were captured using BD Accuri C6 flow cytometer and the data were analysed using FlowJo.

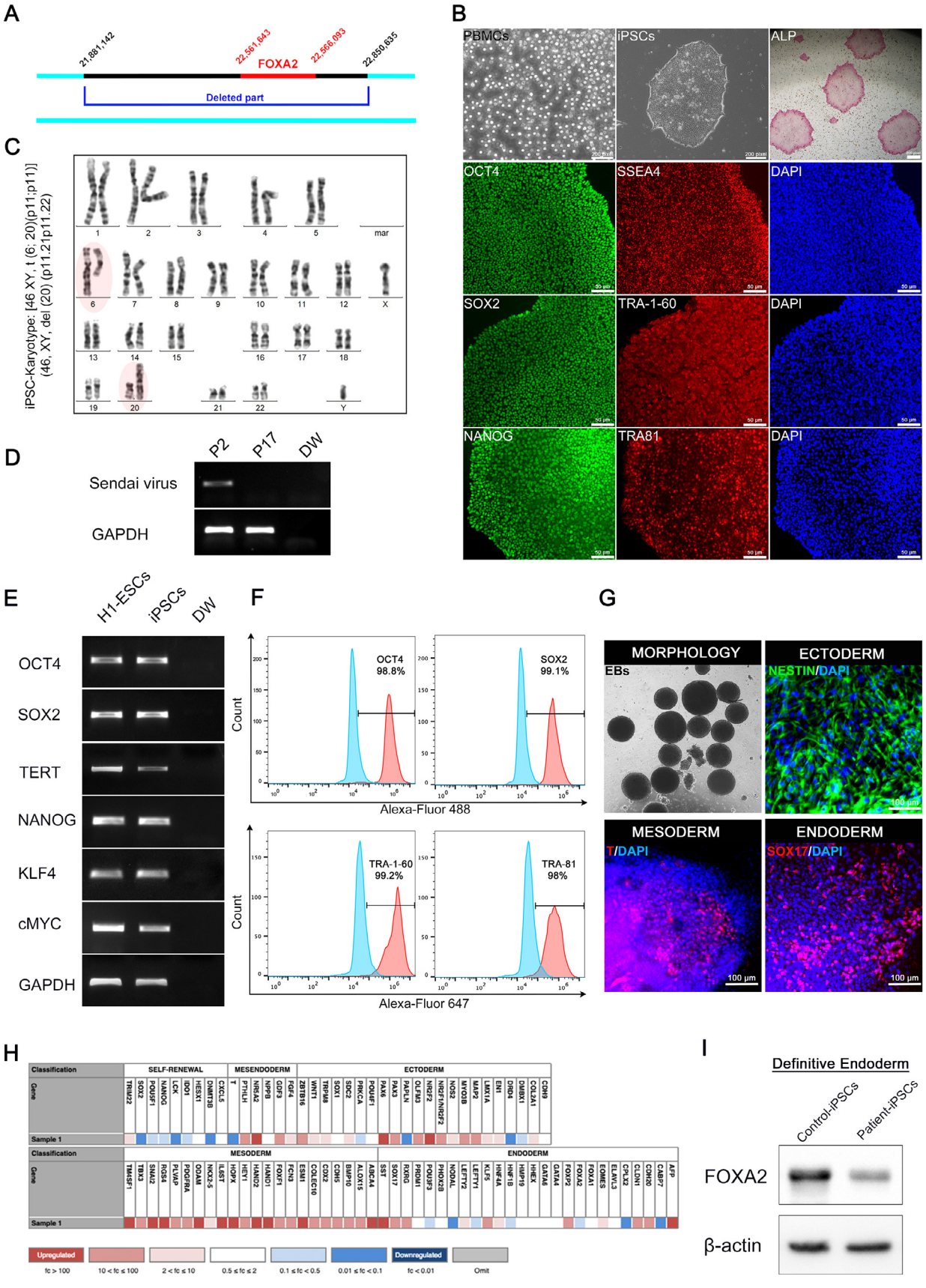
A
21,881,142
22,561,643
FOXA2
22,566,093
22,850,635
Deleted part
B
PBMCs
iPSCs
ALP
OCT4
SSEA4
DAPI
SOX2
TRA-1-60
DAPI
NANOG
TRA81
DAPI
C
iPSC-Karyotype: [46 XY, t (6; 20)(p11;p11)]
(46, XY, del (20) (p11.21p11.22)
D
P2
P17
DW
Sendai virus
GAPDH
E
H1-ESCs
iPSCs
DW
OCT4
SOX2
TERT
NANOG
KLF4
cMYC
GAPDH
F
OCT4
98.8%
SOX2
99.1%
Count
Alexa-Fluor 488
TRA-1-60
99.2%
TRA-81
98%
Count
Alexa-Fluor 647
G
MORPHOLOGY
EBs
ECTODERM
NESTIN/DAPI
MESODERM
T/DAPI
ENDODERM
SOX17/DAPI
100 µm
H
Classification
SELF-RENEWAL
MESENDODERM
ECTODERM
Gene
Sample 1
MESODERM
ENDODERM
Upregulated
Downregulated
fc > 100
10 < fc ≤ 100
2 < fc ≤ 10
0.5 ≤ fc ≤ 2
0.1 ≤ fc < 0.5
0.01 ≤ fc < 0.1
fc < 0.01
Omit
I
Definitive Endoderm
Control-iPSCs
Patient-iPSCs
FOXA2
β-actin

Fig. 1...

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Rabbit anti-SOX2	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Rabbit anti-NANOG	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Mouse anti SSEA4	1:500 (IF)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Mouse anti TRA-1-60	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Pluripotency Markers	Mouse anti TRA-81	1:500 (IF) 1:100 (FACS)	Cell Signaling Technology Cat# 9656, RRID: AB_1658242
Differentiation Markers	Mouse Anti-Human Nestin Monoclonal antibody	1:500	R and D Systems Cat# MAB1259, RRID: AB_2251304
Differentiation Markers	Sox17 mouse monoclonal antibody, clone 2G8	1:2000	OriGene Cat# TA500096, RRID: AB_2255344
Differentiation Markers	Mouse anti-Brachyury	1:1000	Abcam Cat# 140661
Differentiation Markers	Rabbit anti-FOXA2	1:5000 (WB)	Cell Signaling Technology Cat# 3143, RRID: AB_2104878
Secondary antibodies	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21206, RRID: AB_2535792
Secondary antibodies	Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:500	Thermo Fisher Scientific Cat# A10037, RRID: AB_2534013
Western blotting	Mouse anti-β-Actin	1:10,000	Santa Cruz Biotechnology Cat# sc-47778 HRP, RRID: AB_2714189
Western blotting	Peroxidase-AffiniPure Donkey Anti-Rabbit IgG (H + L)	1:10,000	Jackson ImmunoResearch Labs Cat# 711–035–152, RRID: AB_10015282
Primers			
	Target	**Forward/ Reverse primer (5′ − 3′)**	
Pluripotency Markers (RT-PCR)	OCT4	GACAGGGGGAGGGGAGGAGCT AGG/ CTTCCCTCCAACCAGTTGCCCCA AAC	
Pluripotency Markers (RT-PCR)	SOX2	GGGAAATGGGAGGGGTGCAAA AGAGG/ TTGCGTGAGTGTGGATGGGATTG GTG	
Pluripotency Markers (RT-PCR)	cMYC	GCGTCCTGGGAAGGGAGATCCG GAGC/ TTGAGGGGCATCGTCGCGGGAG GCTG	
Pluripotency Markers (RT-PCR)	KLF4	CCCAA TTACCCATCCTTCCT/ ACGATCGTCTTCCCCTCTTT	
Pluripotency Markers (RT-PCR)	NANOG	CATGA GTGTGGATCCAGCTTG/ CCTGAATAAGCAGATCCATGG	
Pluripotency Markers (RT-PCR)	REX1	TCACAG TCCAGCAGGTGTTTG/ TCTTGTCTTTGCCCGTTTCT	
Pluripotency Markers (RT-PCR)	TERT	CCTGCTCAAGCTGACTCGACACCGTG/ GGAAAAGCTGGCCCTGGGGTGGAGC	
Pluripotency Markers (RT-PCR)	DPPA4	GGAGCCGCCTGCCCTGGAAAATTC/ TTTTTCCTGATATTCTATTCCCAT	
Sendi virus	SENDAI VIRUS	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
Mycoplasma primer	Mycoplasma	GGGAGCAAACAGGATTAGATACCCT/TGCACCATCTGTCACTCTGTTAACCTC	
House-Keeping Genes (RT-PCR)	GAPDH	ACGACCACTTTGTCAAGCTCATTTC/ GCAGTGAGGGTCTCTCTCTTCCTCT	

3.4. Karyotyping

At passage 20, KaryoMax colcemid (ThermoFisher Scientific, USA) (100 ng/mL) was added to 70–80% confluent cells for two hours. The cells were trypsinized, collected and treated for 20 min with hypotonic solution (0.75 M KCL, ThermoFisher Scientific, USA) at 37 °C. The cells were fixed using methanol/acetic acid (3:1) solution. Cold cell suspension was dropped onto slides and kept for 1 h at 90 °C. Following trypsinisation, the slides were stained with Giemsa and 20 metaphases were analyzed using mean resolution of 200–300 bands per haploid chromosome set.

3.5. In vitro trilineage spontaneous differentiation and scorecard analysis

Spontaneously differentiated embryoid bodies (EBs) were induced by culturing the dissociated iPSCs in ultra-low attachment plates for 4 days in unconditioned EB medium (DMEM/F12, 20% knockout serum replacement (KSR), 1% NEAA, 1% (v/v) penicillin-streptomycin,1 mM L-glutamine and 0.1 mM 2-mercaptoethanol). The EBs were plated on Matrigel-coated plates for 14–20 days. Trilineage specific markers were examined using immunostaining. Used antibodies are listed in Table 2.

For the scorecard analysis, a wide range examination for the trilineage markers were examined and scored using TaqMan hPSC Scorecard TM kit 96w fast assay (Life Technologies, A15876).

3.6. Short tandem repeat (STR) profiling

STR was performed using the AmpFISTR® Identifiler® Plus PCR amplification Kit (Applied biosynthesis, Life Technologies) following the manufacturer's instructions.

3.7. Mycoplasma detection test

The mycoplasma was routinely examined in the culture medium using specific primers for mycoplasma testing (Table 2).

3.8. RT- PCR amplification

The total RNA was extracted from the cells using RNeasy Plus Mini Kit (Qiagen) and reverse transcribed by Superscript IV, First strand synthesis kit (ThermoFisher Scientific, USA). PCR-Master mix was used to amplify the required products with specific primers for each gene (Table 2).

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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Supplementary materials

Supplementary material associated with this article can be found, in

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