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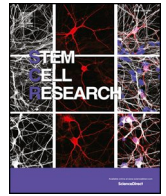
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Generation of two human iPSC lines from patients with maturity-onset diabetes of the young type 2 (MODY2) and permanent neonatal diabetes due to mutations in the *GCK* gene

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ABSTRACT

Heterozygous and homozygous mutations in the *glucokinase* (*GCK*) gene leads to maturity-onset diabetes of the young type 2 (MODY2) and permanent neonatal diabetes (PNDM), respectively. Here, we report the generation of two induced pluripotent stem cell (iPSC) lines, QBRi010-A and QBRi011-A, from patients with MODY2 and PNDM due to mutations in the *GCK* gene (c.437 T > C). The generated iPSC lines displayed pluripotency characteristics, were able to differentiate into the three germ layers, and showed normal karyotypes. These iPSC lines will serve as valuable human cell models for understanding diabetes pathogenesis and developing new therapies for diabetes.

1. Resource Table

Unique stem cell lines identifier	QBRi010-A QBRi011-A
Alternative name(s) of stem cell line	GCK-MODY2 iPSCs (QBRi010-A)
Institution	GCK-PNDM iPSCs (QBRi011-A) Qatar Biomedical research institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, Qatar
Contact information of distributor	Essam M. Abdelalim (emohamed@hbku.edu.qa)
Type of cell line	iPSC
Origin	human
Cell Source	Blood
Clonality	Clonal
Method of reprogramming	Integration-free Sendai virus vector contain OCT3/4, SOX2, c-MYC, and KLF4
Genetic Modification	YES
Type of Modification	Hereditary
Associated disease	Patient 1: (Maturity diabetes of the young type 2 (MODY2)) Patient 2: Permanent neonatal diabetes mellitus (PNDM)
Gene/locus	Gene: <i>GCK</i> Locus: 7p13 Heterozygous mutation: c.437 T > C in exon 4 (Patient 1)

	Homozygous mutation: c.437 T > C in exon 4 (Patient 2)
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	The protocol was approved by the Institutional Review Board (IRB) of Sidra Medicine (no. 1702007608) and QBRI (no. 2018-002)

2. Resource utility

We established two iPSC lines from patients with MODY2 and PNDM due to heterozygous and homozygous mutations in the *GCK* gene (c.437 T > C), respectively. These iPSC lines will serve as human cell models for elucidating underlying mechanism of *GCK*-associated diabetes and developing novel therapies for diabetes.

3. Resource details

Glucokinase (*GCK*) gene encodes an enzyme that phosphorylate

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
QBRIi010-A	QBRIi010-A	Male	54 years old	Egyptian	Heterozygous (<i>GCK</i> , c.437 T > C p.L146P)	Maturity-onset diabetes of the young type 2 (MODY2)
QBRIi011-A	QBRIi011-A	Male	11 years old	Egyptian	Homozygous (<i>GCK</i> , c.437 T > C p.L146P)	Permanent neonatal diabetes mellitus (PNDM)

glucose to glucose-6-phosphate during glycolysis. This is the rate limiting step in glucose metabolism and enables pancreatic β -cells and hepatocytes to respond appropriately to blood glucose level (Gloyn, 2003). Patients with *GCK* mutations have reduced glycolysis, altered intracellular ADP/ATP ratio that affect potassium channel and thus results in impaired insulin secretion (Gloyn, 2003). Heterozygous mutations in *GCK* gene has been reported to cause maturity onset diabetes of young type 2 (MODY2) (Froguel et al., 1992), while homozygous mutations in *GCK* leads to permanent neonatal diabetes mellitus (PNDM) (Njolstad et al., 2001). Here, we generated two iPSC lines, QBRIi010-A and QBRIi011-A, from patients with MODY2 and PNDM, respectively. QBRIi010-A was generated from a 54-year-old male patient with MODY2 (patient 1) due to a heterozygous mutation (c.437 T > C, p.L146P) in the *GCK* gene. Furthermore, QBRIi011-A was generated from an 11-year-old male patient with PNDM (patient 2) due to a homozygous mutation (c.437 T > C, p.L146P) in the *GCK* gene (Table 1). Patient 2 was diagnosed with diabetes at one-day-old and was permanently on insulin treatment (Al-Khawaga et al., 2019). The *GCK* mutations were identified in the patient's sample using whole exome sequencing (WES) and was further confirmed by Sanger sequencing. The mutation (c.437 T > C) in the *GCK* gene leads to the substitution of leucine to proline at position 146 (p.L146P). For iPSC generation, the peripheral blood mononuclear cells (PBMCs) were isolated from patient's blood and transduced with non-integrating Sendai virus expressing OCT3/4, SOX2, c-MYC and KLF4 transcription factors. The generated iPSC-like colonies were picked and expanded for further characterization (Table 2). Sanger sequencing analysis confirmed the *GCK* mutation (c.437 T > C) in the generated iPSC lines (Fig. 1A). The coding sequence used as a reference sequence is the NCBI sequence (NM_000162.4). The iPSC lines, QBRIi010-A and QBRIi011-A, exhibited a typical morphology of human embryonic stem cells (hESCs) (Fig. 1B) and expressed the key pluripotency markers, including OCT4, NANOG, SOX2, SSEA4, TRA-1-60, and TRA-1-81 as examined by

immunocytochemistry (Fig. 1C). The expression of pluripotency markers were further confirmed by RT-PCR and qPCR (Fig. 1D, E). QBRIi010-A and QBRIi011-A silenced the expression of exogenous Sendai viral vector after several passages as confirmed by RT-PCR at passage 22 (Fig. 1F). Karyotype analysis of both iPSC lines and the patient's blood samples showed normal karyotype with a cytogenetic balanced pericentric inversion within chromosome 9 (46,XY,inv(9)(p11q13) (Supplementary Fig. 1A-B), which is a normal variant with no clinical significance (Shaffer et al., 2013). Both cell lines were able to form embryoid bodies (EBs) upon spontaneous differentiation and expressed specific markers of the three germ layers, including NESTIN (endoderm), brachury (T) (mesoderm), and SOX17 (ectoderm) (Fig. 1G, H). The generated cell lines passed the scorecard analysis with high scores for the three germ layers and lost the pluripotency expression upon spontaneous differentiation (Fig. 1I). RT-PCR analysis confirmed that these iPSC lines are not contaminated with mycoplasma (Supplementary Fig. 1C, D). The origin of the iPSC lines were confirmed by short tandem repeat (STR) profiling, which confirmed the same genetic identity of the patient's PBMCs.

4. Materials and methods

4.1. Cell culture and reprogramming

Blood samples were collected from the donors with informed consent and PBMCs were isolated using Ficoll-Paque (Sigma-Aldrich). The cells were cultured in StemPro-34 complete medium (Gibco) supplemented with FLT3 (100 ng/ml), IL6 (20 ng/ml), TPO (100 ng/ml), SCF (100 ng/ml) for four days before reprogramming. The cells were reprogrammed using CytoTune-iPS 2.0 Sendai reprogramming kit (Thermo Fisher Scientific). Established iPSC clones were cultured onto plates coated with Geltrex and fed with StemFlex medium (Thermo Fisher Scientific).

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal for both lines	Fig. 1 panel B
Phenotype	Qualitative analysis	Assess staining/expression of pluripotency markers: OCT4, SOX2, NANOG, SSEA4, TRA-1-60, TRA-1-81, TERT, REX1, DPPA4, c-MYC, and KLF4	Fig. 1 panel C, and D
	Immunocytochemistry		
	RT-PCR		
	Quantitative analysis RT-RT-qPCR	OCT4, SOX2, NANOG, TERT, REX1 are positive for both cell lines and are similar to hESCs	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	Both 46XY with mean resolution of 300	Supplementary Fig. 1 panels A, and B
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 15 loci, 100% matched	N/A Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous and homozygous mutations (c.437 T > C)	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR	Supplementary Fig. 1 panels C, and D
	Sendai virus	Negative by RT-PCR	Fig. 1 panel F
Differentiation potential	Embryoid body formation and Scorecard	The embryoid body formed and express NESTIN, BRACHYURY and SOX17 (immunostaining and RT-PCR) as well as the expression of NEUROD1 (RT-PCR)	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

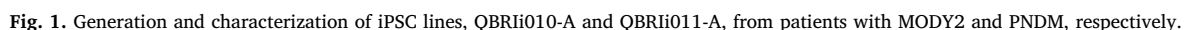


Table 3
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:500 (IF)	Cell Signaling Technology Cat# 9656, RRID:AB_1658242
Pluripotency Markers	Rabbit anti-SOX2	1:500 (IF)	Cell Signaling Technology Cat# 9656, RRID:AB_1658242
Pluripotency Markers	Rabbit anti-NANOG	1:500 (IF)	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)	Cell Signaling Technology Cat# 9656, RRID:AB_1658242
Pluripotency Markers	Mouse anti TRA-81	1:500 (IF)	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# ab140661
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
Primers			
	Target	Forward/Reverse primer (5'–3')	
Pluripotency Markers (RT-PCR/RT-qPCR)	<i>OCT4</i>	GACAGGGGGAGGGGAGGAGCT AGG/CTTCCCTCCAACCAAGTTGCCCA AAC	
Pluripotency Markers (RT-PCR/RT-qPCR)	<i>SOX2</i>	GGGAAATGGGAGGGGTGCAAA AGAGG/TTGCGTGAGTGTGGATGGGATTG GTG	
Pluripotency Markers (RT-PCR)	<i>c-MYC</i>	GCGTCCTGGGAAGGGAGATCCG GAGC/TTGAGGGGCATCGTCGCGGGAG GCTG	
Pluripotency Markers (RT-PCR)	<i>KLF4</i>	CCCAA TTACCCATCCTTCCT/ACGATCGTCTCCCTCTTT	
Pluripotency Markers (RT-PCR/RT-qPCR)	<i>NANOG</i>	CATGA GTGTGGATCCAGCTTG/CCTGAATAAGCAGATCCATGG	
Pluripotency Markers (RT-PCR/RT-qPCR)	<i>REX1</i>	TCACAG TCCAGCAGGTGTTTG/TCTGTCTTTGCCGTTTCT	
Pluripotency Markers (RT-PCR/RT-qPCR)	<i>TERT</i>	CCTGCTCAAGCTGACTCGACACCGTG/ GGAAAAGCTGGCCCTGGGGTGGAGC	
Pluripotency Markers (RT-PCR)	<i>DPPI4</i>	GGAGCCGCTGCCCTGGAAATTC/TTTTCCTGATATTCTATCCCAT	
Sendi virus	<i>SENDI VIRUS</i>	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTAAGAGATATGTATC	
Ectodermal differentiation	<i>NEUROD1</i>	CGAATTGGTGTGGCTGTATTC/GGAGAGGAAAGAAGTGCTAAGG	
Mesodermal differentiation	<i>BRACHYURY</i>	GCCCTCTCCCTCCCTCCACGCACAG/ CGGCGCCGTTGCTCACAGACCACAGG	
Endodermal differentiation	<i>SOX17</i>	TCCTGGAGGAGCTAAGGAAA/GCCACTTCCCAAGGTGTAAA	
House-Keeping Genes (RT-PCR)	<i>GAPDH</i>	ACGACCACCTTTGTCAAGCTCATTTG/GCAGTGAGGGTCTCTCTCTCTCT	
Targeted mutation analysis/ sequencing	<i>GCK</i>	GATCTCCCTTCTGAGCACATG/TCCTGAGGAATAGCTTGGCTTG	
Mycoplasma primer	<i>Mycoplasma</i>	GGGAGCAAACAGGATTAGATACCCT/ TGACCATCTGTCACTCTGTAACTCTC	

4.2. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde in 0.1 M PBS for 20 min, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) in 0.1 M PBS and blocked with 6% bovine serum albumin. The cells were incubated with primary antibodies at 4 °C overnight (Table 3), then washed with 0.3% Tween-20 in 0.1 M PBS and incubated with the secondary antibodies (Table 3) for 1 h at room temperature. Images were acquired using an inverted fluorescence microscope (Olympus IX 53).

4.3. Sanger sequencing

Genomic DNA was extracted using quick extract genomic DNA extraction buffer (epicenter). The region of *GCK* spanning the mutation was amplified using PCR-Master mix (ThermoFisher Scientific) and specific primers (Table 3). The PCR products were purified and sequenced.

4.4. Karyotype analysis

The cells were processed using standard protocols for G-banding. Briefly, to arrest the cells at the metaphase, they were treated with 100 ng/ml KaryoMax colcemid (ThermoFisher Scientific). The arrested cells were further exposed to 0.75 M KCL hypotonic solution (ThermoFisher Scientific) for 20 min at 37 °C and then fixed with methanol: glacial acetic acid (3:1). 20 metaphases were karyotyped for each sample.

4.5. Gene expression analysis

Total RNA was isolated using direct-zol RNA MiniPrep kit (Zymo Research) according to the manufacturer's instructions and complementary DNA was synthesized using SuperScript IV First-Strand Synthesis System (Thermo Fisher Scientific). quantitative PCR (qPCR) was performed using GoTaq qPCR Master (Promega) with the primers listed in Table 3, using H1-hESCs as a positive control and gene expression was normalized to GAPDH.

4.6. Embryoid body (EB) formation and scorecard analysis

iPSCs were detached as small clumps and plated in ultra-low attachment plates in DMEM/F12 medium supplemented with 20% Knockout Serum Replacement, 1 mM L-glutamine, 1% non-essential amino acids, 0.1 mM 2-mercaptoethanol, 1% (v/v) penicillin–streptomycin for 4 days. EBs were then plated on geltrex coated plates for 14 days and examined for the expression of all germ layers markers using RT-PCR and immunostaining. Scorecard analysis was performed using the TaqMan hPSC Scorecard assay (Life Technologies, A15876). TaqMan master mix was added to the diluted cDNA. 10 µl was loaded per well into hPSC Scorecard plate and run on a QuantStudio7 Flex Real-Time PCR system (Applied Biosystems). The results were analysed using an online TaqMan hPSC Scorecard analysis software (<https://www.thermofisher.com/qa/en/home/life-science/stem-cell-research/taqman-hpsc-scorecard-panel/scorecard-software.html>).

4.7. Short tandem repeat profiling (STR)

STR was performed using AmpFISTR Identifier Plus PCR amplification Kit (Applied biosynthesis, Life Technologies) according to the manufacturer's instructions.

4.8. Mycoplasma detection test

The cells were regularly checked for the absence of mycoplasma contamination in the culture media using PCR with the primers listed in Table 3.

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.

Acknowledgment

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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.2020.101991>.

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