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Lab Resource: Multiple Cell Lines

Generation of three induced pluripotent stem cell lines (UQACi003-A, UQACi004-A, and UQACi006-A) from three patients with *KRT5* epidermolysis bullosa simplex mutations

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A B S T R A C T

Heterozygous mutations within Keratin 5 (*KRT5*) are common genetic causes of epidermolysis bullosa simplex (EBS), a skin fragility disorder characterized by blisters, which appear after minor trauma. Using CytoTune®Sendai virus, we generated three human induced pluripotent stem cell (iPSC) lines from three EBS patients carrying respectively the single heterozygous mutations in *KRT5*, c.449 T > C, c.980 T > C, and c.608 T > C. All lines display normal karyotype, expressed high levels of pluripotent markers, and can differentiate into derivatives of the three germ layers. These iPSCs are helpful for a better understanding of the EBS pathogenesis and developing novel therapeutic approaches.

Resource Table:

Unique stem cell lines identifier	1- UQACi003-A 2- UQACi004-A 3- UQACi006-A
Alternative name(s) of stem cell lines	iPSC-EBS2 iPSC-EBS9 iPSC-EBS26
Institution	Université du Québec à Chicoutimi, Saguenay, QC, Canada
Contact information of distributor	Catherine Laprise, Catherine.Laprise@uqac.ca
Type of cell lines	iPSC
Origin	Human skin cells
Additional origin info required *for human ESC or iPSC*	UQACi003-A, Age: 7, Sex: Male, Ethnicity: Canadian UQACi004-A, Age: 34, Sex: Male, Ethnicity: Canadian UQACi006-A, Age: 51, Sex: Female, Ethnicity: Canadian
Cell Source	Human fibroblasts
Clonality	Clonal
Associated disease	Epidermolysis bullosa simplex
Gene/locus	Keratin 5 gene (*KRT5*), locus 12q13.13, Autosomal dominant mutations NM_002275 (*KRT5*): UQACi003-A: Heterozygous (c.449 T > C) UQACi004-A: Heterozygous (c.980 T > C) UQACi006-A: Heterozygous (c.608 T > C)
Date archived/stock date	October 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/UQACi003-A https://hpscreg.eu/cell-line/UQACi004-A https://hpscreg.eu/cell-line/UQACi006-A
Ethical approval	Le comité d'éthique de la recherche avec des êtres humains de l'Université du Québec à Chicoutimi, Canada (Approval number 602.162.05)

1. Resource utility

The three iPSC lines (UQACi003-A, UQACi004-A, and UQACi006-A) generated from three EBS patients carrying pathogenic variants within *KRT5 in exon 1, exon 4 and exon 2 respectively* will be genetically

Abbreviations: EBS, Epidermolysis bullosa simplex; KRT5, Keratin 5; KRT14, Keratin 14; iPSC, Induced pluripotent stem cell; FACS, Fluorescence-activated cell sorting; RT-PCR, Reverse transcription polymerase chain reaction; EB, Embryoid bodies; STR, Short tandem repeat; FGF, Fibroblast growth factor; DPBS, Dulbecco's Phosphate-Buffered Saline.

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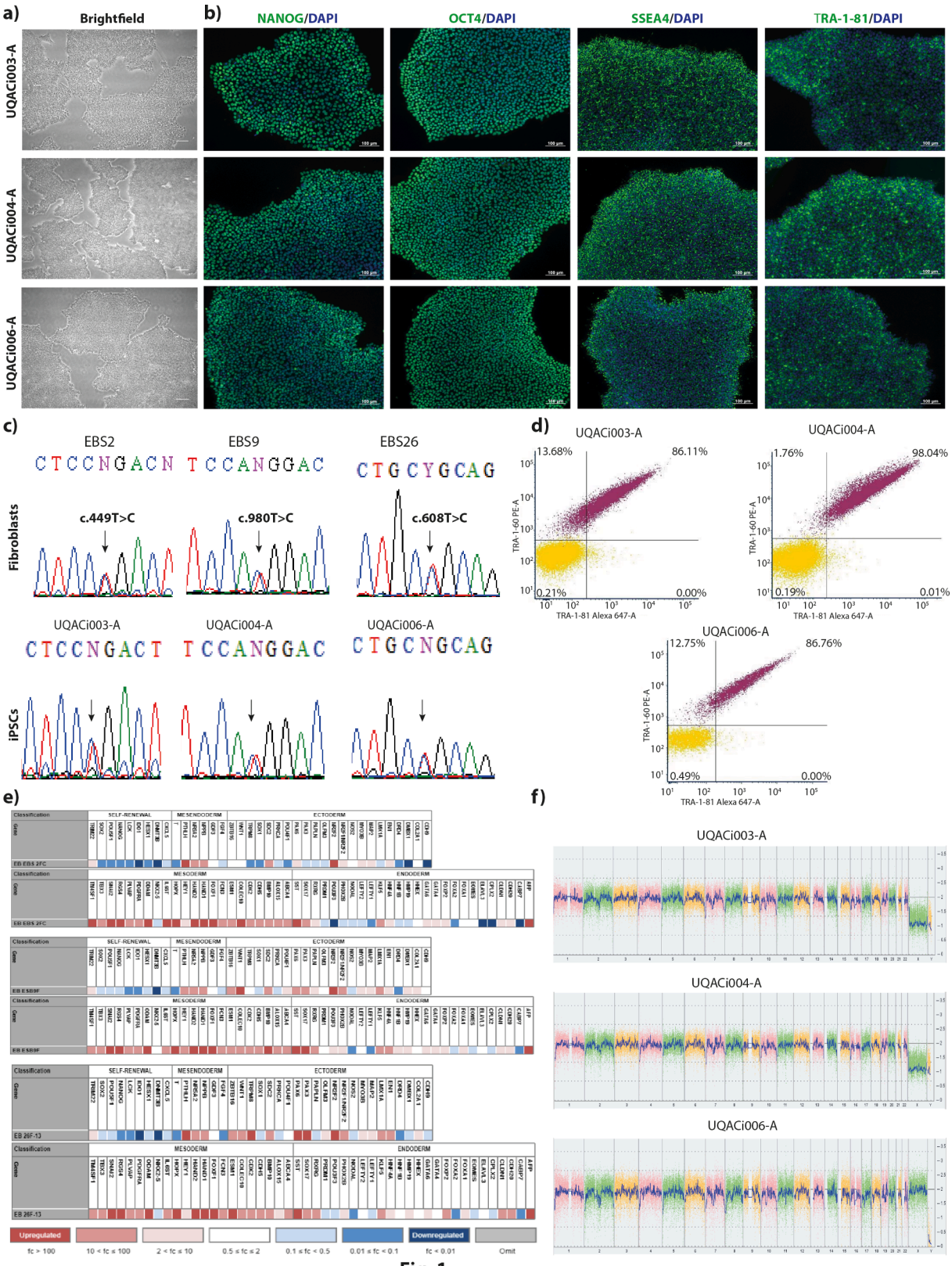

Fig. 1.

Fig. 1. Generation and functional characterization of human induced pluripotent stem cells (iPSCs) from patients with epidermolysis bullosa simplex. a) Typical embryonic stem cell-like colony morphology for UQACi003-A, UQACi004-A, and UQACi006-A derived from fibroblasts reprogramming. b) Immunofluorescence analysis showed expression of pluripotent markers OCT4, NANOG, TRA1-81 and the surface marker SSEA4 (scale bars: 100 μm). c) Electropherograms showed the presence of the mutations in the patient's fibroblasts and in the iPSCs lines. d) Expression analysis of pluripotent markers TRA-1-81 and TRA-1-60 by flow cytometry analysis showed expression in 86–98% of cells. e) Scorecard analysis showed the expression of several genes of the three germ layers (endoderm, mesoderm and ectoderm). f) KaryoStat assay analysis showed normal karyotype for the three iPSCs lines.

corrected using the CRISPR/Cas9 technology and differentiated into keratinocytes that will be used to synthetize skin tissues serving as autografts for affected patients.

2. Resource details

Epidermolysis bullosa simplex (EBS) is a rare skin disorder usually caused by mutations in the keratin intermediate filament keratin 5 (*KRT5*) or keratin 14 (*KRT14*) genes (Coulombe et al., 1991). This skin disease is characterized by cutaneous fragility and blisters formation and there is currently no effective treatment beyond supportive care to ameliorate patients' quality of life. The generation of the patient-specific iPS cells are promising models for the fields of personalized therapies and regenerative medicine (Okita et al., 2007). In this study, we successfully reprogrammed, three iPSC lines from skin fibroblasts of three EBS patients heterozygous for pathogenic variations within *KRT5*. UQACi003-A is from a patient aged of seven years (EBS2) and presented severe EBS symptoms manifesting by blisters in many skin regions. This patient holds the c.449 T > C (p.Leu150Pro) within exon 1. UQACi004-A derived from a mildly affected patient (EBS9) aged of 34 years and carrying the c.980 T > C (p.Met327Thr) within exon 4. p.Leu150Pro and p.Met327Thr variations were previously published by our team (Bchetnia et al., 2012). UQACi006-A is from a mildly affected 51 year old patient (EBS26) expressing the mutation c.608 T > C (p.Leu 203Pro) within exon 2. We report the identification of this pathogenic variation in this patient for the first time herein (Fig. 1**c**) and we highlight that p. Leu 203Pro variation was previously described in the literature only once before in a severely affected patient (Cho et al., 2014). EBS patient fibroblasts were reprogrammed using Sendai virus containing the four Yamanaka factors. The iPSC clones showed typical human embryonic stem cell morphology forming dense, sharp-edged colonies (Fig. 1**a**). In the three iPSC lines, pluripotency markers expression was confirmed by immunofluorescence staining for markers NANOG, OCT3/4, SSEA-4, and TRA-1-81 (Fig. 1**b**), by fluorescence-activated cell sorting (FACS) for the pluripotency makers TRA-1-60 and TRA-1-81 showing expression in 86–98% of cells (Fig. 1**d**), and by reverse transcription polymerase chain reaction (RT-PCR) for markers OCT3/4, NANOG, REX1, DNMT3b and hTERT (**Supplementary Fig. S1a**). Four weeks after reprogramming, Sendai virus was not detected by RT-PCR indicating the successful activation of endogenous pluripotent genes (**Supplementary Fig. S1b**). The generated iPSC clones formed embryoid bodies (EBs) and spontaneously differentiated into the three germ layers (endoderm, mesoderm and ectoderm) as validated by scorecard analysis (Fig. 1**e**). The *KRT5* mutations c.449 T > C, c.980 T > C, and c.608 T > C carried by the studied patients were identified in the parental fibroblasts and in the generated iPSCs by Sanger sequencing (Fig. 1**c**) Karyotype was assessed by KaryoStat assay and was normal with no clonal abnormalities (Fig. 1**f**). Cell identities were verified by comparing short tandem repeat (STR) analyses of fibroblasts and their derived iPSCs for nine Short Tandem Repeat (STR) markers with amelogenin for sex determination. All iPSC lines were mycoplasma free (**Supplementary Fig. S1c**).

In summary, we generated three iPSC lines for three EBS patients heterozygous for pathogenic variations within *KRT5* gene and these lines are a suitable cellular model to develop novel therapies for this cutaneous disease.

3. Materials and methods

3.1. Reprogramming

Dermal fibroblasts were cultured in the fibroblast media consisted of DMEM with 10% FBS and 1% Penicillin/Streptomycin (Thermo Fisher Scientific) at 37 °C, 5% CO2. 3×10^5 cells, at passage 2, were plated on irradiated mouse embryonic fibroblasts (Amsbio) and reprogrammed to iPSCs by the genome integration-free SeV virus kit (CytoTune™-2.0, ThermoFisher Scientific) according to the manufacturer's instructions.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel a
Phenotype	Qualitative analysis Immunocytochemistry	Positive staining for NANOG, OCT4, SSEA4, and TRA-1-81,	Fig. 1 panel b
	Quantitative analysis RT-PCR and Flow cytometry	Expression of pluripotent markers OCT4, NANOG, REX1, DNM3TB, HTERT, TRA-1-81 and TRA-1-60	Fig. 1 panel d and Supplementary Fig. S1a
Genotype	Molecular karyotyping using Karyostat™	46XY for UQACi003-A and UQACi004-A 46XX for UQACi006-A	Fig. 1 panel f
Identity	STR analysis	10 sites tested, all matched	With authors
Mutation analysis (IF APPLICABLE)	Sequencing	UQACi003-A, UQACi004-A, and UQACi006-A are heterozygous for the mutations c.449 T > C, c.980 T > C, and c.608 T > C in *KRT5* respectively	Fig. 1 panel c
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative	Supplementary Fig. S1c
Differentiation potential	Scorecard	Expression of genes of all three germ layers	Fig. 1 panel e
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

Culturing media is KnockDMEM/F-12, 20% KnockOut Serum Replacement, Glutamax, 1%MEM Non-Essential Amino Acids, 55 μM 2-Mercaptoethanol and basic fibroblast growth factor (bFGF) at 10 μg/ml. The iPSCs were cultured and maintained in Matrigel (Corning) with mTeSR1 medium (StemCell Technologies™) at 37 °C and 5% CO2. ROCK inhibitor (Y27632, Selleck Chemicals) was added for 24 h after passaging to improve cell survival and attachment. When clones with hESC like appearance (line H1) reached a sufficient size, they were manually picked, passaged and expanded. The iPSCs were passaged every 3–4 days using 0,5 mM EDTA.

3.2. Mutation confirmation

Genomic DNA was extracted from primary fibroblasts at passage 2 and iPSCs using QuickExtract™ DNA Extraction Solution (Epicentre). PCR amplification was performed using primers described in Table 2. The PCR products were sequenced on an ABI 3730xL Genetic Analyzer (Applied Biosystems).

3.3. Immunofluorescent staining

The pluripotency status of the three iPSCs lines was evaluated by immunostaining for NANOG, OCT4, SSEA4, and TRA-1-81. Briefly, the iPSCs were fixed for 15 min at room temperature using 4%

Table 2
Reagents details.

		Antibodies	
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-OCT3/4	1:500	Santa Cruz Cat# sc-5279, RRID: AB_628051
	Mouse anti-NANOG	1:2000	Millipore Cat# MABD24, RRID: AB_11203829
	Mouse anti-SSEA4	1:100	ThermoFisher Scientific Cat# 41-4000, RRID: AB_2533506
	Mouse anti-TRA1-60	1:100	StemCell Technologies Cat# 60064, RRID: AB_2686905
	Mouse anti-TRA1-81	1:500	ThermoFisher Scientific Cat# MA1-24, RRID: AB_2356706
Secondary antibodies	Rabbit anti-mouse IgG (H + L), Alexa-Fluor 488	1:500	ThermoFisher Scientific Cat# A-11001, RRID: AB_2534069
		Primers	
	Target	Size of band (pb)	Forward/Reverse primer (5′-3′)
Sendai virus detection (PCR)	SeV	181	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS	528	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG
	KLF4	410	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA
	C-MYC	532	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG
Pluripotency Markers (RT-PCR)	NANOG	225	CCTGGAACAGTCCCTTCTATAAC TCACTCATCTTCACACGTCTTC
	OCT4	1070	GTGGAGGAAGCTGACAACAA CAGGTTTTCTTTCCCTAGCT
	DNM3TB	230	TGCTGCTCACAGGGCCCGATACTTC TCCTTTCGAGCTCAGTGCACCACAAAAC
	hTERT	200	TGTGCACCAACATCTACAAG GCGTTCTTGGCTTTCAGGAT
	REX1	220	CGCAATCGCTTGTCCTCAGAG GCTCTCAACGAACGCTTTCCCA
Reference gene	ACTB	329	GGACTTCGAGCAAGAGATGG AGCACTGTGTTGGCGTACAG
Targeted mutation analysis (Sanger sequencing)	*KRT5* (exon 1)	575	CTTGTGGAGTGGGTGGCTAT ATTAGACTGGCAGCTGGGAA
	KRT5 (exon2)	418	TGGGAGGCACCTTAGTGAGT TACCTTCCATGGACACCTCC
	KRT5 (exon4)	531	TTGAGTAGGGTGACGGTG CATGACGACACTAAGAAT

paraformaldehyde and washed with Dulbecco's Phosphate-Buffered Saline (DPBS). They were permeabilized with 0.1% Triton™X-100, and then incubated in blocking solution containing 1% BSA, 0.3% Triton™X-100 in DPBS at room temperature. Cells were then incubated overnight at 4 °C with the primary antibodies in 1% BSA. Afterwards, they were incubated with the secondary antibodies for 1 h at room temperature (Table 1). DAPI was used to stain cell nuclei and images were taken using the fluorescent microscope Zeiss Axio Observer Microscope.

3.4. Flow cytometry

IPSCs were dissociated into single cells with accutase, count, and incubated with a fixable viability stain (BD Horizon, Cat# 565388; dilution 1:1000) for 30 min on ice. Cells were then washed two times with stain buffer (BD Phamingen Cat#554656) and incubated with TRA-1-81 and TRA-1-60 antibodies and their isotype controls, 30 min at room temperature, in obscurity. Analysis was performed using the flow cytometer BD FACSMelody.

3.5. RT-PCR analysis

In the generated iPSCs, pluripotency expression of markers OCT3/4, NANOG, REX1, DNMT3b and hTERT was validated by RT-PCR (at passage 15) as well as the absence of Sendai virus vectors SEV, KOS, KLF4, and C-MYC (at passage 6). Briefly, total RNA was isolated from iPSCs and H1-hESC using Direct-zol™ RNA miniprep and converted into cDNA using the Quantitect Reverse transcription kit. We assessed the RNA integrity and yield using Agilent BioAnalyser RNA 2100 and on 2% agarose gel. PCR was performed using HotStarTaq plus DNA polymerase (Qiagen) and expression of pluripotency genes was compared to it levels in H1-hESC. All primers used in this study are listed in Table 2.

3.6. Spontaneous differentiation in vitro

iPSCs were harvested with accutase and plated in non-adherent dishes in EB medium consisting of DMEM/F12, 20% KnockOut™ Serum Replacement 1% non-essential amino acids and 1% GlutaMAX™(ThermoFisher Scientific), 0.1 mM 2-mercaptomethanol and 50 μM rock inhibitor Y-27632. After 8 days, spontaneous forming embryoid bodies (EB) were transferred in suspension, onto a gelatin coated plate and cultured for another 8 days. Markers characterizing the three germ layers were assessed by an assay using the scorecard™ Kit 384w (Applied Biosystems) following manufacturer's instructions (Fergus et al., 2016).

3.7. Karyotype analysis

Each iPSC line was analyzed at passage 5 using the KaryoStat™ assay (Thermo Fisher Scientific).

3.8. STR analysis

Short tandem repeat (STR) analysis of ten loci was performed on the patients' fibroblasts and the established iPSC lines by Genome Québec laboratories.

3.9. Mycoplasma detection

Mycoplasma detection on cell culture was performed using the Venor®GeM Mycoplasma PCR Detection Kit (Cederlane).

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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Appendix A. Supplementary data

a) Pluripotency expression of markers OCT3/4, NANOG, REX1, DNMT3b and hTERT in the iPSC lines by RT-PCR. b) Absence of expression of Sendai virus vectors SEV, KOS, KLF4, and C-MYC by RT-PCR. c) Absence of mycoplasma contamination using the Venor®GeM Mycoplasma PCR Detection Kit (Cederlane). PCR products were loaded on 2% TBE 1X agarose gel. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2022.102726.

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