



Lab Resource: Single Cell Line

Generation of an induced pluripotent stem cell line CSSi015-A (9553), carrying a point mutation c.2915C > T in the human calcium sensing receptor (CaSR) gene

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ABSTRACT

Familial Hypocalciuric Hypercalcemia (FHH1) is a rare autosomal dominant disease with low penetrance, caused by inactivating mutations of the calcium-sensing receptor (CaSR) gene, characterized by significant hypercalcemia, inappropriately normal serum PTH levels and a low urinary calcium level. Human induced pluripotent stem cells (hiPSCs) from a patient carrying a previously identified heterozygous mutation, a p.T972M amino acid substitution in cytoplasmic tail of CaSR, were produced using a virus, xeno-free and non-integrative protocol.

1. Resource table

Unique stem cell line identifier	CSSi015-A(9553)
Alternative name(s) of stem cell line	FC 17 cl D1
Institution	Fondazione IRCCS Casa Sollievo della Sofferenza
Contact information of distributor	Jessica Rosati; j.rosati@css-mendel.it
Type of cell line	iPSC
Origin	human
Additional origin info required for human ESC or iPSC	Age:38 Sex: Male Ethnicity if known: Caucasian/Italian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Non integrating episomal vectors
Genetic Modification	yes
Type of Genetic Modification	congenital
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	qRT-PCR
Associated disease	Familial benign Hypocalciuric Hypercalcemia (FHH)
Gene/locus	CaSR c.2915C > T

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Unique stem cell line identifier	CSSi015-A(9553)
Date archived/stock date	Luglio 2018
Cell line repository/bank	https://hpscereg.eu/user/cellline/edit/CSSi015-A
Ethical approval	CE: Prot n 230/16, RIF CE 4047, Università degli studi di Roma "La Sapienza", Dipartimento di Scienze Neurologiche, Psichiatriche e Riabilitative dell'età evolutiva.

2. Resource utility

Familial Hypocalciuric Hypercalcemia (MIM#145980) is a rare genetic disorder caused by inactivating mutations of the Calcium Sensing receptor (CaSR) gene. Variable phenotypic expression, due to the high number of CaSR identified mutations, makes difficult a possible phenotype-genotype association. The derivation of iPSCs permits to study this disease *in vitro*. [Table 1](#).

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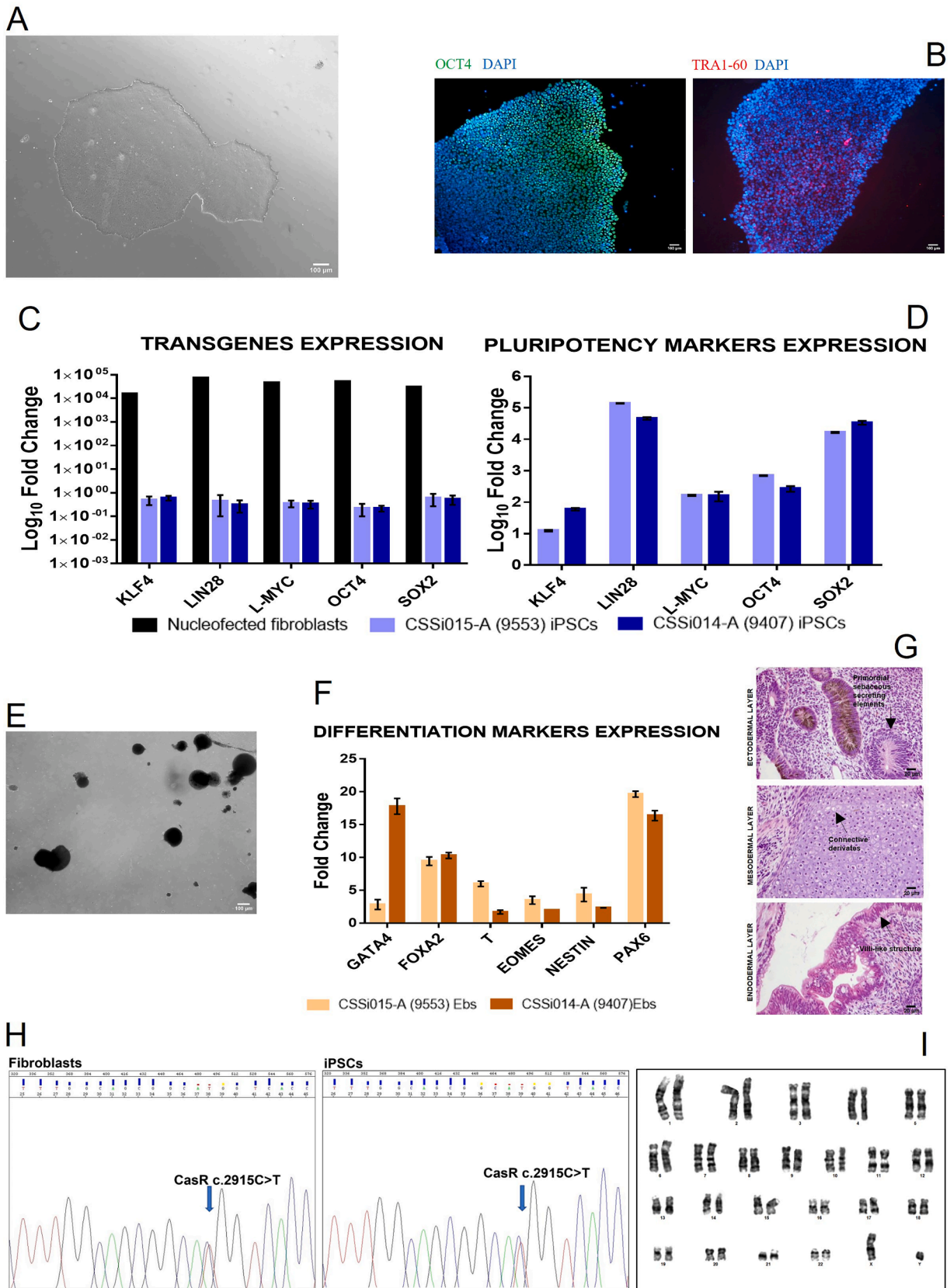


Fig. 1.

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit anti-OCT4; Mouse anti TRA-1-60	1:100 1:100	Life technologies (A13998); Life technologies (411000)	RRID: AB_2534182; RRID: AB_2533494.
Secondary antibodies	anti-Rabbit AlexaFluor 488; anti-Mouse AlexaFluor 594	1:1000 1:1000	Invitrogen (A11034); Invitrogen (A21422)	RRID: AB_2576217; RRID: AB_2535844
	Primers Target	Size of band	Forward/Reverse primer (5'-3')	
SyBr green Primers used for qPCR	eOCT4 eKLF4 eLIN28	83 bp 112 bp 205 bp	Fwd: CAT TCAAAC TGA GGTAAG GG Rev: TAG CGTAAA AGG AGCAAC ATA G	
Episomal genes	eL-MYC eSOX2	80 bp 66 bp	Fwd: CCA CCTCGC CTT ACACAT GAA GA Rev: TAG CGTAAA AGG AGCAAC ATA G Fwd: AGC CATATG GTA GCCTCA TGT CCG C Rev: TAG CGTAAA AGG AGCAAC ATA G Fwd: GGC TGAGAA GAG GATGGC TAC Rev: TTT GTTTGA CAG GAGCGA CAA T Fwd: TTC ACATGT CCC AGCACT ACC AGA Rev: TTT GTTTGA CAG GAGCGA CAA T	
SyBr green Primers used for qPCR	OCT4 LIN28 l-MYC	143 bp 129 bp 142 bp	Fwd: CCC CAGGGC CCC ATTTTG GTA CC Rev: ACC TCAGTT TGA ATGCAT GGG AGAGC	
Pluripotency Markers (qPCR)	SOX2	80 bp	Fwd: AGC CAT ATG GTA GCC TCA TGT CCG C Rev: TCA ATT CTG TGC CTC CGG GAG CAG GGT AGG Fwd: GCG AACCCA AGA CCCAGC CCT GCTCC Rev: CAG GGGTC TGC TCGCAC CGT GAT G Fwd: TTC ACATGT CCC AGCACT ACC AGA Rev: TCA CATGTG TGA GAGGGG CAG TGTGC	
House-Keeping Genes (qPCR)	β -ACTIN	203 bp	Fwd: GGC ATCCTC ACC CTGAAG TA Rev: GGG GTGTTG AAG GTCTCA AA	
TaqMan primers used for qPCR	NESTIN PAX6 T	50 bp 76 bp 132 bp	Hs04187831_g1 Hs00240871_m1 Hs00610080_m1	
Differentiation markers	EOMES GATA4 FOXA2 β -ACTIN	81 bp 68 bp 66 bp 171 bp	Hs00172872_m1 Hs00171403_m1 Hs00232764_m1 Hs 99999903_m1	
	CASR	167 bp		

Table 2 (continued)

Antibodies used for immunocytochemistry/flow-cytometry			
Antibody	Dilution	Company Cat #	RRID
e.g. Targeted mutation analysis/sequencing			Fwd: CACAGCAGCAACGATCTCAG Rev: GGG GTGTTG AAG GTCTCA AA

4.5. Immunofluorescence staining

Cells, at XII passage, were fixed using 4 % paraformaldehyde, incubated with Blocking Buffer (PBS containing 20 % Normal Goat Serum, 0.1 % Triton X-100) for 30 min at room temperature and then with primary antibodies, Table 2, O/N at 4 °C. After washing, Alexa Fluor 594- and/or Alexa Fluor 488-conjugated secondary antibodies were added 1 h at RT. Cellular nuclei were counterstained with DAPI. Microphotographs were taken using a Nikon C2 fluorescence microscope and NIS Elements 1.49 software.

4.6. Karyotype analysis

Pluripotent cells (VII passage) were cultured in T 25 flasks coated with Matrigel in Nutristem medium for 2–3 days. Cells were treated with a 0.1 μ g/mL COLCEMID solution (Thermo Fisher Scientific) for 60 min at 37 °C, 30 mM KCl in 10 %FBS at 37 °C for 6 min and coldfresh-made 3:1 ethanol:acetic acid solution. Karyotype analysis was carried out on GTG-banded metaphases. Fifteen metaphases were counted.

4.7. Mutation analysis

Genomic DNA was extracted from iPSCs and fibroblasts (VI passage) using ReliaPrep™ Blood gDNA Miniprep System. CaSR exon 7 was amplified by PCR using Forward: 5- CACAGCAGCAACGATCTCAG-3, Reverse: 5-CGTATCGCTGCTTTTCTGGG –3' primers. The amplicon (product size: 167 bp) was sequenced by BigDye terminator v.3.1 Cycle Sequencing kit on ABI 3130XL Genetic Analyzer.

4.8. STR analysis

PCR amplification of 16 distincts STRs (D3S1358, TH01, D21S11, D18S51, D10S1248, D1S1656, D2S1338, D16S539, D22S1045, vWA, D8S1179, FGA, D2S441, D12S391, D19S433, SE33) was performed using Power Plex ® ESX 17 Fast System (Promega), PCR products were separated on an ABI Prism 3130 DNA sequencer and analyzed by Gene Mapper IDX v3.2 (Applied Biosystems).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jessica Rosati reports financial support was provided by Ministry of Health.

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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.2023.103023>.

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