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Lab Resource: Multiple Stem Cell Lines

Generation of induced pluripotent stem cell lines from a Crisponi/Cold induced sweating syndrome type 1 individual

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A B S T R A C T

Cytokine receptor like factor 1 (*CRLF1*) is the gene implicated, when mutated, in Crisponi syndrome/cold-induced sweating syndrome type 1 (CS/CISS1). Here, we report the establishment of induced pluripotent stem cell lines (iPSCs) from fibroblasts of a Turkish CS/CISS1 individual with a homozygous variant in *CRLF1* (c.708_709delinsT; p.[Pro238Argfs*6]). This variant is the most frequent variant associated to CS/CISS1 in the Turkish population. These patient derived iPSC lines show all pluripotency markers, a normal karyotype and the ability to differentiate into the three germ layers.

Resource Table:

Unique stem cell line identifier	UKMi001-A UKMi001-B UKMi001-C
Alternative name(s) of stem cell line	CRLF1-iPSC-C1 (UKMi001-A) CRLF1-iPSC-C4 (UKMi001-B) CRLF1-iPSC-C6 (UKMi001-C)
Institution	Muenster University Children's Hospital Department of General Pediatrics Albert-Schweitzer-Campus 1 D-48149 Muenster, Germany
Contact information of distributor	Dr. rer. nat. Insa Buers insa.buers@ukmuenster.de
Type of cell line	iPSC
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Transgene free (Sendai virus)
Multiline rationale	Isogenic clones
Genetic Modification	YES
Type of Modification	Hereditary mutation
Associated disease	Crisponi syndrome/cold-induced sweating syndrome 1
Gene/locus	CRLF1/chr19p12-13.1
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	November 2018
Cell line repository/bank	N/A
Ethical approval	The study was approved by the ethics committee of Muenster University (number: 2017-523-f-s). Patient gave written informed consent for the study

1. Resource utility

The CS/CISS1 specific iPSC lines will be a powerful new resource for modelling cell-specific deficits in CS/CISS1 and can be used not only to understand the cellular consequences of disease-causing variants but also for the development of new therapeutic strategies for CS/CISS1.

2. Resource details

CS/CISS1 is characterized by severe thermoregulatory and orofacial/laryngeal muscular defects (Crisponi 1996). Pathogenic variants in *CRLF1* are the most frequent cause for CS/CISS1. *CRLF1* consists of nine coding exons and encodes the soluble cytokine receptor CRLF1. CRLF1 together with cardiotrophin-like cytokine factor 1 (CLCF1) forms an intracellular heterodimer complex that activates ciliary neurotrophic factor receptor (CNTFR) expressing cells after secretion. Mutated CRLF1 either is partially or no longer secreted resulting in inadequate activation of the CNTFR pathway and finally in disturbed or

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Table 1
Summary of lines

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UKMi001-A	CRLF1-iPSC-C1	Female	9 years	European	Homozygous	Crisponi syndrome/cold-induced sweating syndrome1
UKMi001-B	CRLF1-iPSC-C4	Female	9 years	European	Homozygous	Crisponi syndrome/cold-induced sweating syndrome1
UKMi001-C	CRLF1-iPSC-C6	Female	9 years	European	Homozygous	Crisponi syndrome/cold-induced sweating syndrome1

reduced differentiation of motor neurons. Here, we establish the generation of three CRLF1-iPSC clones (CRLF1-iPSC-C1, CRLF1-iPSC-C4, CRLF1-iPSC-C6) from a 9-year-old female carrying the homozygous *CRLF1* variant c.708_709delinsT (Table 1). This variant is localized in *CRLF1* exon 5, resulting in a premature stop and in the expression and partial secretion of a truncated CRLF1 protein (p.[Pro238Argfs*6]).

Patient fibroblasts, obtained by nasal brushing, were reprogrammed by transduction of Sendai viral vectors containing the four Yamanaka factors *Octamer binding transcription factor 3/4* (*OCT3/4*), *Sex determining region Y-box 2* (*SOX2*), *Kruppel-like factor 4* (*KLF4*) and *c-MYC* using CytoTune™-iPS 2.0 Reprogramming System (Life Technologies/Thermofisher Scientific). The expression of stem cell markers RNA in all CRLF1-iPSC-clones was confirmed by quantitative PCR (q-PCR; *OCT 3/4, SOX2, NANOG*; Fig. 1A) and reverse transcription PCR (RT-PCR; e.g. *OCT3/4, KLF4, c-MYC, SOX2, FGF4, TERT*; Supplementary Fig. 1). All CRLF1-iPSC-clones showed typical stem cell morphology and immunocytochemical staining confirmed the presence of OCT3/4, and KLF4 (Fig. 1B). Additionally, cell surface markers such as stage-specific embryonic antigen-4 (SSEA4), Tra-1-60 and Tra-1-81, as well as the transcription factor NANOG, were detectable in all three CRLF1-iPSC-clones (Fig. 1B). G-banding karyotype analysis showed normal diploid 46,XX karyotype (Fig. 1C). The three CRLF1-iPSC-clones harbour the homozygous *CRLF1* c.708_709delinsT variant confirmed by Sanger sequencing (Fig. 1D). To confirm pluripotency of the CRLF1-iPSC clones they were differentiated into the three germ layers (ectoderm, mesoderm or endoderm) using the STEMdiff™-Trilineage Differentiation Kit (Stem Cell Technologies, Germany). Typical markers for endoderm (FOX2A), mesoderm (Brachyury) and ectoderm (ßIII-Tubulin) were detectable after differentiation using confocal microscopy (Fig. 1E). Additionally, cells showed no contamination with mycoplasma (Supplementary Fig. 2). Parental fibroblasts and the CRLF1-iPSC-clones shared alleles with 100% match validated by analysis of eight high polymorphic and autosomal microsatellites (D3S1358, D10S1248, D2S441, D13S317, D16S539, D18S51, D19S433, D21S11, D12S391) plus DXS8060, and AMEL for gender determination (available by request).

3. Materials and methods

3.1. Extraction of fibroblasts

Fibroblasts of a CS/CISS1 individual were obtained by transnasal brush biopsy (Cytobrush Plus; Medscand Medical) and suspended in RPMI medium (Gibco). After washing cells were resuspended in DMEM-F12 medium containing 2% Ultroser-G (Cytogen) and plated on collagen-coated flasks. After 3 weeks, collagen was resolved with collagenase type IV and fibroblasts were passaged. The study was approved by the ethics committee of Muenster University (number: 2017-523-f-s).

3.2. Generation of iPSC-clones

Generation of CRLF1-iPSC-clones was performed using the CytoTune™-iPS 2.0 Reprogramming System (Life Technologies/Thermofisher Scientific) according to the manufacturer's specifications. Briefly: At a density of 50-80% confluency, fibroblasts were transduced using the CytoTune 2.0 Sendai vectors for 24h. After transduction fibroblast media was changed every other day. Seven days later transduced cells were seeded on MEFs in fibroblast medium. Medium was switched to iPSC media after 24h and changed three times a week. iPSC colonies with typical morphology were picked and cultured on Matrigel (Corning)-coated plates using mTeSR Plus medium (Stem Cell Technologies).

3.3. In vitro differentiation

The iPSCs were harvested with gentle cell dissociation reagent (Stem Cell Technologies) and plated for trilineage differentiation according to the STEMdiff™-Trilineage Differentiation Kit protocol (Stem Cell Technologies).

3.4. Mycoplasma detection

The mycoplasma test was performed by mycoplasma detection kit MycoSPY® (Biontex Laboratories) according to the manufacturer's instructions.

3.5. Karyotyping and microsatellite analysis

Metaphases for karyotyping based on conventional G-banding analysis were prepared according to standard procedures (Barch et al., 1997). For microsatellite analysis, we used 11 polymorphic microsatellite markers (Table 1). PCR reactions with one fluorescent-labelled primer for each marker were performed using described touchdown protocols (Hecker et al., 1996). PCR-products were analyzed on an ABI3730 sequencer (Thermo Fisher Scientific Inc.) and evaluated using GeneMarker software version 1.51 (Softgenetics LLC). For sex determination a polymorphic sequence in intron 3 of both amelogenin genes (Akane et al., 1991) was analyzed by standard PCR.

3.6. RT- and qPCR

Isolation of total RNA was performed by phenol-chloroform precipitation. cDNA was synthesized by reverse transcription using Superscript™ III reverse transcriptase (Invitrogen™ Life Technologies). PCRs were performed using TAQ DNA polymerase (Qiagen) in a Mastercycler epGradientS (Eppendorf). Primer sequences used are listed in Table 2. qPCR was performed using CFX Touch Real Time PCR Detection System (Bio-Rad) and the iQ Syber Green Supermix (Bio-Rad) following our standard protocols (Buers et al., 2016). Specific primers are listed in Table 2. Gene expression level analysis was performed in triplicate and normalized to 18 sRNA.

3.7. Immunofluorescence staining

Immunofluorescence staining was performed following our standard protocols (Buers et al., 2016). Primary antibodies (Table 3) were incubated overnight at 4°C followed by incubation with secondary antibodies (Table 3) at RT for one hour and by DAPI incubation for 10 min. After mounting samples were examined with a Zeiss Apotome Axiovert 200 or LSM880 (Zeiss) and processed with AxioVision v.4.8 and Adobe CS4.

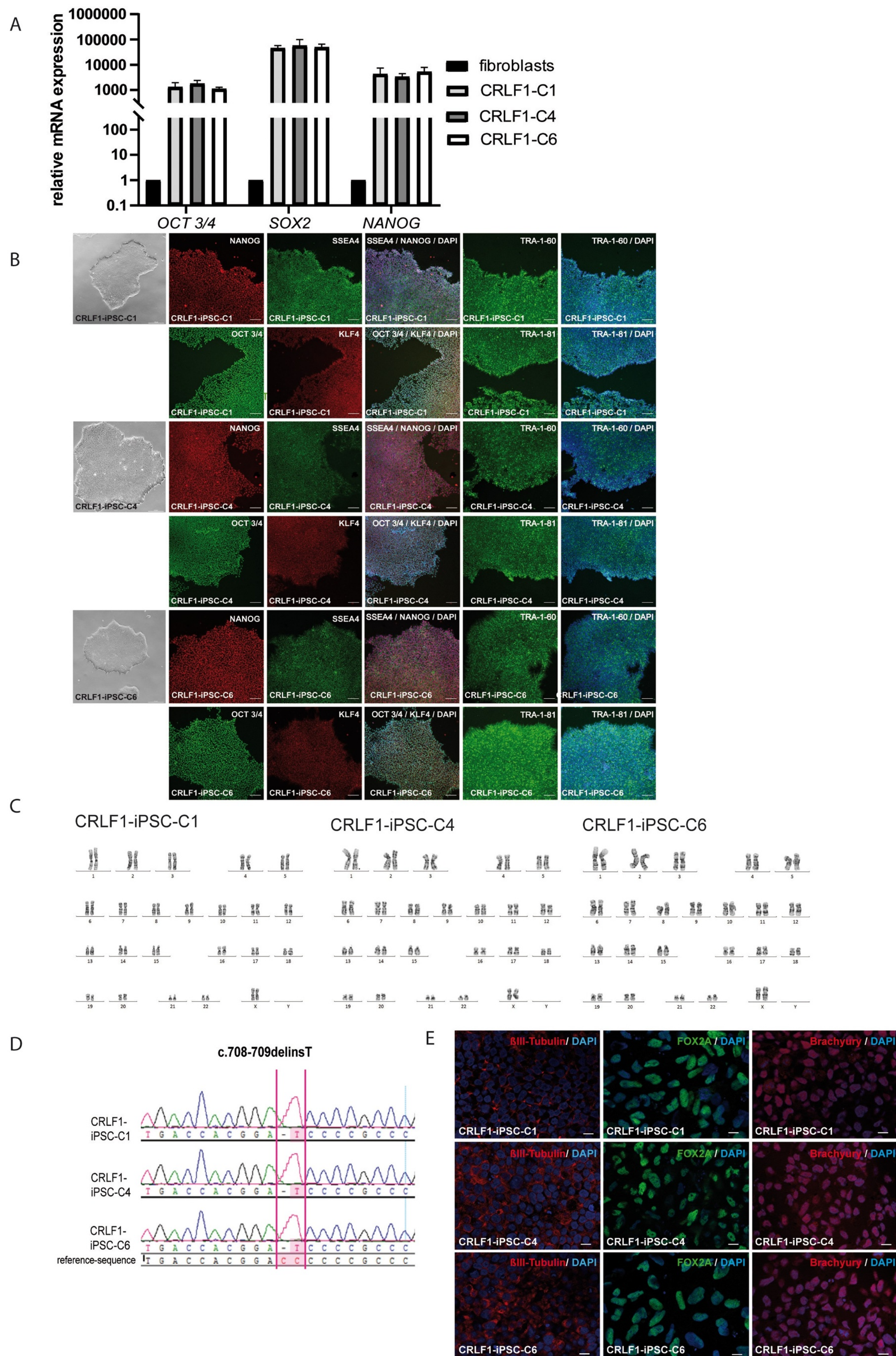

Fig. 1. Characterization of iPSC

Table 2
Characterization and validation

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel B
Phenotype	Qualitative analysis by Immunocytochemistry	OCT 3/4, KLF 4, NANOG, Tra-1-60, Tra-1-81, SSEA4.	Fig. 1 panel B
	Quantitative analysis by qPCR and RT-PCR	Positive for OCT3/4, NANOG, SOX2	Fig. 1 panel A
Genotype	Karyotype (G-banding) and resolution	Normal, 46, XX Resolution 250-300	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR) OR	Not performed	N/A
	STR analysis	11 loci sites (D3S1358, D2S441, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11,DXS8060, AMEL) tested and all matched	Available with authors
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	c.708_709delinsT	Fig. 1 panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative	Suppl. Fig 1B
Differentiation potential	In-vitro differentiation with STEMdiff^{TM}-Trilineage Differentiation Kit	Endoderm: Forkhead Box A2 (FOXA2), Mesoderm: Brachyury, Ectoderm: βIII-Tubulin	Fig. 1 panel E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Negative	Not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-Human OCT3/4 Antibody, Clone 3A2A20	1:100	STEMCELL Technologies Cat#60093, RRID:AB_2801346
	Mouse anti-Human SSEA-4 Antibody, Clone MC-813-70	1:100	STEMCELL Technologies Cat#60062, RRID:AB_2721031
	Mouse anti-Human TRA-1-60 Antibody	1:100	BD Bioscience Cat#560173, RRID:AB_1645379
	Mouse anti-Human TRA-1-81 Antibody, Clone TRA-1-81	1:100	STEMCELL Technologies Cat#01556, RRID:AB_1118559
	Rabbit anti-KLF4	1:100	Cell Signalling Technology Cat#4038, RRID:AB_2265207
	Goat anti-NANOG	1:100	R & D Systems Cat#AF1997, RRID:AB_355097
Differentiation Markers	Rabbit anti-FOXA2	1:200	Abcam Cat#193864
	Goat anti-Brachyury	1:200	R & D Systems Cat# AF2085, RRID:AB_2200235
	Mouse anti ßIII-Tubulin antibody	1:200	Abcam Cat# ab78078, RRID:AB_2256751
Secondary antibodies	Donkey anti-Mouse IgG, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607
	Donkey anti-Rabbit IgG, Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-10040, RRID:AB_2534016
	Donkey anti-Goat IgG, Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-11056, RRID:AB_2534103
	Donkey anti-Mouse IgG, Alexa Fluor 546	1:1000	Jackson ImmunoResearch Labs Cat# 715-166-150, RRID:AB_2340816
	Donkey anti-Rabbit IgG, Alexa Fluor 488	1:1000	Jackson ImmunoResearch Labs Cat#711-546-152, RRID:AB_2340619
Primers			
	Target	Forward/Reverse primer (5′-3′)	
Pluripotency markers (qPCR)	*OCT3/4*	GACAGGGGGAGGGGAGGAGCTAGG/CTTCCCTCCAAC CAGTTGCCCCAAAC	
	SOX2	GGGAAATGGGAGGGGTGCAAAAGAGG/TTGCGTGAG TGTGGATGGGATTGGTG	
	NANOG	ACC CCA GCC TTT ACT CTT CC/CTG GAT GTT CTG GGT CTG GT	

(*continued on next page*)

Table 3 (*continued*)

Antibodies used for immunocytochemistry/flow-citometry	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers (RT-PCR)	Endo OCT3/4	GACAGGGGGAGGGGAGGAGCTAGG/CTTCCCTCCAAC CAGTTGCCCCAAAC	
	NANOG	CAGCCCCGATTCTTCCACCAGTCCC/CGGAAGATTCCC AGTCGGGTTCACC	
	REX1	CAGATCCTAAACAGCTCGCAGAAT/GCGTACGCAAAT TAAAGTCCAGA	
	DPPA4	GGAGCCGCCTGCCCTGGAAAATTC/TTTTTCCTGATA TTCTATTCCCAT	
	DPPA2	CCGTCCCCGCAATCTCCTTCCATC/ATGATGCCAACA TGGCTCCCGGTG	
	DNMT3B	TGCTGCTCACAGGGCCCGATACTTC/TCCTTTCGAGCT CAGTGCACCACAAAAC	
	GABRB3	CCTTGCCCAAAATCCCCTATGTCAAAGC/GTATCGCCA ATGCCGCCTGAGACCTC	
	GAL	TGCGGCCCGAAGATGACATGAAACC/CCCAGGAGGCTC TCAGGACCGCTC	
	IFITM1	CCCCAAAGCCAGAAGATGCACAAGGAG/CGTCGCCAA CCATCTTCCTGTCCCTAG	
	GRB7	TCCAGCCCCACAGCAGCATCAACTACC/CCGGGTTGA AGGTGGCTTTGACTGCTC	
	CD9	GTGCATGCTGGGACTGTTCTTCGGCTTC/CACGCCCCC AGCCAAACCACAGCAG	
	BRIX	CACCACGGTATCATCCCAAAAGCCAACC/ACGCCGATG CATGTTTGGTGACTGGTAG	
	GDF3	CTTATGCTACGTAAAGGAGCTGGG/GTGCCAACCCAG GTCCCGGAAGTT	
	FGF4	CTACAACGCCTACGAGTCCTACA/GTTGCACCAGAA AAGTCAGAGTTG	
	Endo SOX2	GGGAAATGGGAGGGGTGCAAAAGAGG/TTGCGTGAG TGTGGATGGGATTGGTG	
	TDGF1	CTGCTGCCTGAATGGGGGAACCTGC/GCCACGAGGTGC TCATCCATCACAAGG	
	Endo c-MYC	GCGTCCTGGGAAGGGAGATCCGGAGC/TTGAGGGGC ATCGTCGCGGGAGGCTG	
	LEFTB	CTTGGGGACTATGGAGCTCAGGGCGAC/CATGGGCAG CGAGTCAGTCTCCGAGG	
	NODAL	GGGCAAGAGGCACCGTCGACATCA/GGGACTCGGTGG GGCTGGTAACGTTTC	
	hTERT	CCTGCTCAAGCTGACTCGACACCGTG/GGAAAAGCT GGCCCTGGGGTGGAGC	
	UTF1	CCGTCGCTGAACACCGCCCTGCTG/CGCGCTGCCCAG AATGAAGCCCAC	
	KLF4 endo	ACGATCGTGGCCCCGGAAAAGGACC/TGATTGTAGTGC TTTCTGGCTGGGCTCC	
Housekeeping Gene	18sRNA	AAACGGCTACCACATCCAA/CCTCCAATGGATCCTCGT TA	
Sequencing Primer	CRLF1-Exon 5	TGTAAAACGACGGCCAGTGAAAACAGAGGCAGGTTC CA/ CAGGAAACAGCTATGACCGGACAGTGAGGACAA GGTCAG	

4. Variant identification

DNA was isolated with QIAamp DNA Mini Kit (Qiagen) according to manufactures instructions. PCR products were sequenced by Sanger sequencing. Primers are listed in Table 2.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101820.

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