Stem Cell Research 46 (2020) 101855

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

Crisponi syndrome/cold-induced sweating syndrome type 2: Reprogramming of CS/CISS2 individual derived fibroblasts into three clones of one iPSC line

Insa Buers[a,*,1], Lara Schöning[a,1], Niki Tomas Loges[a], Yvonne Nitschke[a], Inga Marlena Höben[a], Albrecht Röpke[b], Laura Crisponi[c], Heymut Omran[a], Frank Rutsch[a]

[a] Department of General Pediatrics, Muenster University Children's Hospital, Muenster, Germany
[b] Institute of Human Genetics, University of Muenster, Muenster, Germany
[c] Istituto di Ricerca Genetica e Biomedica, Consiglio Nazionale delle Ricerche, Cagliari, Italy

A B S T R A C T

Crisponi syndrome/cold-induced sweating syndrome type 2 (CS/CISS2) is a rare disease with severe dysfunctions of thermoregulatory processes. CS/CISS2 individuals suffer from recurrent episodes of hyperthermia in the neonatal period and paradoxical sweating at cold ambient temperatures in adolescence. Variants in *CLCF1 (cardiotrophin-like-cytokine 1)* cause CS/CISS2. Here, we summarize the generation of three clones of one stem cell line (iPSC) of a CS/CISS2 individual carrying the *CLCF1* variant c.321C > G on both alleles. These patient derived iPSC clones show a normal karyotype, several pluripotency markers, and the ability to differentiate into the three germ layers.

1. Resource Table

Unique stem cell line identifier	UKMi002-A UKMi002-B UKMi002-C
Alternative name(s) of stem cell line	CLCF1-iPSC-C2 (UKMi002-A) CLCF1-iPSC-C4 (UKMi002-B) CLCF1-iPSC-C19 (UKMi002-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 type 2
Gene/locus	CLCF1/chr11q13.2
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.

2. Resource utility

Although the first *cardiotrophin-like cytokine-1 (CLCF1)* variants were identified more than one decade ago, the pathomechanism underlying Crisponi syndrome/cold-induced sweating syndrome type 2 (CS/CISS2, MIM: #610313) is still poorly understood. The iPSCs generated from a CS/CISS2 individual provide an ideal resource for exploring the pathogenesis of CS/CISS2 and also for improvement of therapeutic options for CS/CISS2.

3. Resource details

CS/CISS2 is an autosomal recessive disease characterized by recurrent episodes of hyperthermia and facial muscle contractions, which lead to breathing and feeding difficulties in the neonatal period. Additionally, CS/CISS2 individuals show camptodactyly and sometimes develop scoliosis. In adolescence, cold-induced sweating may occur when ambient temperature reaches below 20 °C (Hahn and Boman, 2016). CS/CISS2 is caused by variants in the *CLCF1* gene. So far, four pathogenic *CLCF1* variants are described in the literature (Hahn and

* Corresponding author.
[1] These authors should be considered joint first authors.

https://doi.org/10.1016/j.scr.2020.101855
Received 20 April 2020; Received in revised form 3 May 2020; Accepted 25 May 2020
Available online 01 June 2020

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UKMi002-A	CLCF1-iPSC-C2	Male	11 years	European	Homozygous	Crisponi Syndrome/cold-induced sweating syndrome 2
UKMi002-B	CLCF1-iPSC-C4	Male	11 years	European	Homozygous	Crisponi Syndrome/cold-induced sweating syndrome 2
UKMi002-C	CLCF1-iPSC-C19	Male	11 years	European	Homozygous	Crisponi Syndrome/cold-induced sweating syndrome 2

Boman, 2016). CLCF1 forms a heterodimer with cytokine receptor like factor 1 (CRLF1) which after secretion activates the JAK/STAT signaling pathway by binding to the ciliary neurotrophic factor receptor (CNTFR). Mutated CLCF1 causes insufficient activation of CNTFR signaling and finally results in inadequate differentiation of motor neurons. Here, we describe the generation of three CLCF1-iPSC clones (CLCF1-iPSC-C2, CLCF1-iPSC-C4, CLCF1-iPSC-C19, Table 1) of one iPSC line from an 11-year-old boy carrying the unpublished homozygous *CLCF1* variant c.321C > G. This variant is localized in *CLCF1* exon 3 and causes a premature stop of translation (p.Tyr107*).

Reprogramming of CS/CISS2 fibroblasts was performed using the CytoTune™-iPS 2.0 Sendai Reprogramming System (Life Technologies/Thermofisher Scientific). Sendai virus-based reprogramming consists of 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. CLCF1 mutated iPSC clones show strong expression of stem cell markers such as *OCT 3/4, SOX2, NANOG*, confirmed by quantitative PCR (Fig. 1A). In addition to the characteristic stem cell morphology, CLCF1 mutated iPSC clones display typical localization of OCT3/4 and KLF4 (Fig. 1B). Furthermore, iPSC specific cell surface markers such as stage-specific embryonic antigen-4 (SSEA4), Tra-1-60 and Tra-1-81 were detectable as well as the transcription factor NANOG in the CLCF1 mutated iPSC clones by immunocytochemical staining (Fig. 1B). All clones showed no significant differences in proliferation and morphology. A normal diploid 46, XY karyotype was confirmed by G-banding karyotype analysis (Fig. 1C). Sanger sequencing verified the presence of the homozygous *CLCF1* variant c.321C > G in the CLCF1 mutated iPSC clones (Fig. 1D). To demonstrate pluripotency, CLCF1-iPSC clones were differentiated into the three germ layers using the STEMdiff™-Trilineage Differentiation Kit (Stem Cell Technologies). Characteristic markers for endoderm (FOX2A), mesoderm (Brachyury) and ectoderm (βIII-Tubulin) were detectable after differentiation using confocal microscopy (Fig. 1E). Furthermore, no contamination with mycoplasma was detectable in culture media of iPSCs (Supplementary Fig. 1). Parental fibroblasts and the CLCF1 mutated iPSC clones shared alleles with 100% match validated by analysis of eight high polymorphic and autosomal microsatellites (D3S1358, D19S433, D13S317, D16S539, D18S51, D21S11, D2S441, D12S391) plus DXS8060, and AMEL for gender determination (available on request).

4. Materials and methods

4.1. Extraction of fibroblasts

CLCF1 mutated fibroblasts were obtained by transnasal brush biopsy (Cytobrush Plus; Medscand Medical). Transfer of fibroblasts into RPMI medium (Gibco) was followed by washing of the cells. Then fibroblasts were resuspended in DMEM-F12 medium containing 2% Ultroser-G (Cytogen) and cultivated on collagen-coated flasks. 3 weeks later, fibroblasts were passaged after resolving collagen with collagenase type IV. The study was approved by the ethics committee of Muenster University (number: 2017-523-f-s).

4.2. Generation of iPSC clones

CLCF1 mutated fibroblasts were reprogramed using the CytoTune™-iPS 2.0 Sendai Reprogramming System (Life Technologies/Thermofisher Scientific) according to the manufacturer's instructions. Transduction of fibroblasts was performed using the CytoTune 2.0 Sendai vectors for 24 h followed by media change every other day. After seven days transduced fibroblasts were plated on irradiated MEFs in fibroblast medium. 24 h later, medium was aspirated and substituted by iPSC media which then was changed three times a week. iPSC colonies with characteristic iPSC morphology were transferred to matrigel (Corning)-coated plates and cultured in mTeSR Plus medium (Stem Cell Technologies).

4.3. In vitro differentiation

Gentle cell dissociation reagent (Stem Cell Technologies) was used for the collection of CLCF1 mutated iPSCs. Afterwards cells were plated for trilineage differentiation according to the STEMdiff™-Trilineage Differentiation Kit instructions (Stem Cell Technologies).

4.4. Mycoplasma detection

Mycoplasma detection kit MycoSPY® (Biontex Laboratories) was used according to the manufacturer's instructions for the detection of mycoplasma.

4.5. Karyotyping and microsatellite analysis

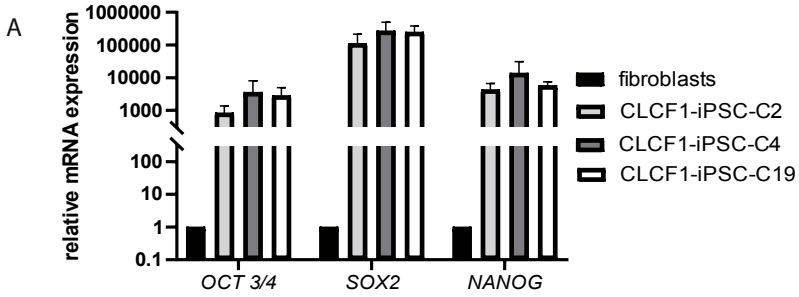
Conventional G-banding analysis was used to prepare metaphases for karyotyping (Barch et al., 1997). For microsatellite analysis, we used 11 polymorphic microsatellite markers (Table 2). PCR reactions with one fluorescent-labelled primer for each marker were performed using described touchdown protocols (Hecker and Roux, 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.

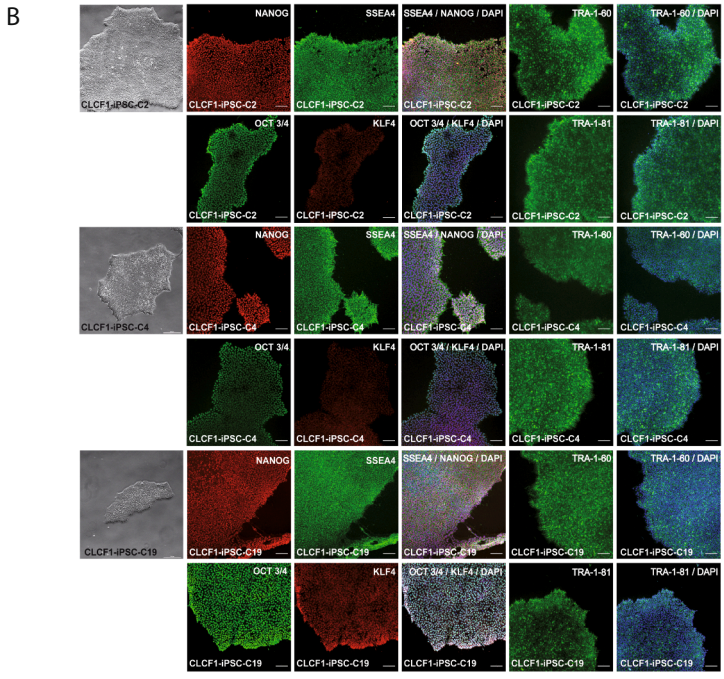
4.6. qPCR studies

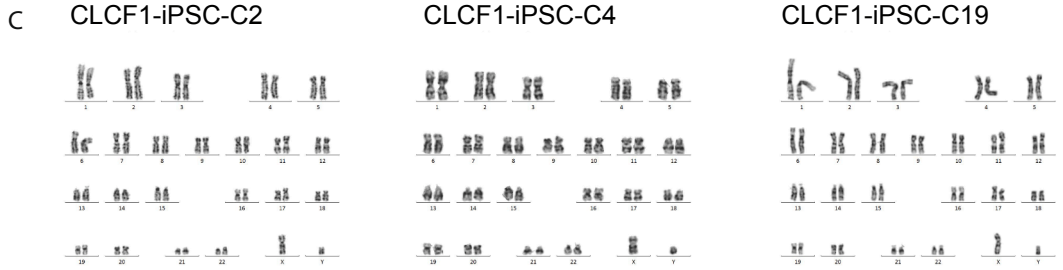
Total RNA was isolated by phenol–chloroform precipitation. cDNA was synthesized using Superscript™ III reverse transcriptase (Invitrogen™ Life Technologies). qPCR studies were done following our standard procedures (Buers et al., 2016) in a CFX Touch Real Time PCR Detection System (Bio-Rad) using iQ Syber Green Supermix (Bio-Rad). Specific primers are listed in Table 3. Gene expression level analysis was performed in triplicate and normalized to *18sRNA*.

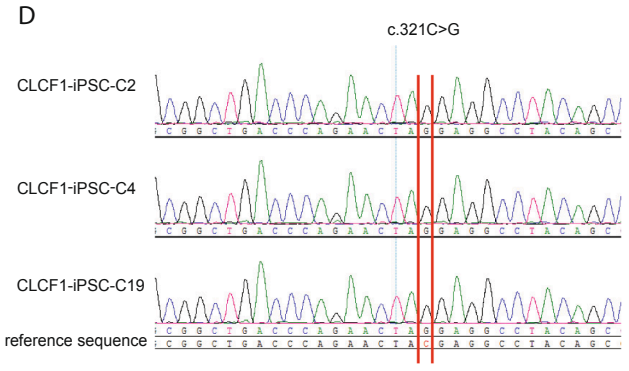
4.7. Immunocytochemical staining

Immunocytochemical staining was performed following our standard procedures (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 (Table 3).









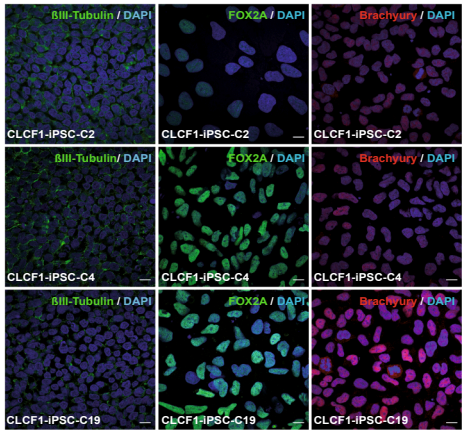


Fig. 1. Characterization of CLCF1 mutated iPSC clones.

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	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)	Not performed	N/A
	STR analysis	10 loci sites (D3S1358, D19S433, D13S317, D16S539, D18S51, D21S11, D2S441, D12S391, DXS8060, AMEL) tested and all matched	Available with authors
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	c.321C > G	Fig. 1 panel D
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR, negative	Suppl. Fig. 1
Differentiation potential	*In-vitro* differentiation with STEMdiff™-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 authors
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-cytometry

	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		
	Mouse anti-Human TRA-1–60 Antibody		STEMCELL Technologies Cat#60062, RRID:AB_2721031
	Mouse anti-Human TRA-1–81 Antibody, Clone TRA-1–81	1:100	
	Rabbit anti-KLF4		BD Bioscience Cat#560173, RRID:AB_1645379
	Goat anti-NANOG		STEMCELL Technologies Cat#01556, RRID:AB_1118559
		1:100	
			Cell Signalling Technology Cat#4038, RRID:AB_2265207
		1:100	R & D Systems Cat#AF1997, RRID:AB_355097
		1:100	
		1:100	
Differentiation markers	Rabbit anti-FOXA2	1:200	Abcam Cat#193864
	Goat anti-Brachyury		
	Mouse anti-beta III Tubulin antibody	1:200	R & D Systems Cat# AF2085, RRID:AB_2200235
			Abcam Cat# ab78078, RRID:AB_2256751
		1:200	
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		
	Donkey anti-Goat IgG, Alexa Fluor 546	1:1000	Thermo Fisher Scientific Cat# A-10040, RRID:AB_2534016
	Donkey anti-Mouse IgG, Alexa Fluor 546		
	Donkey anti-Rabbit IgG, Alexa Fluor 488	1:1000	Thermo Fisher Scientific Cat# A-11056, RRID:AB_2534103
		1:1000	Jackson ImmunoResearch Labs Cat# 715–166-150, RRID:AB_2340816
		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	ACCCCAGCCTTTACTCTTCC/CTGGATGTTCTG GGTCTGGT
Housekeeping gene	*18sRNA*	AAACGGCTACCACATCCAA/CCTCCAATGGATCCTCGTTA
Sequencing primer	*CLCF1*-Exon 3	CAGGTATCTCCTTGGTGGTGA/AGGAGTCCAAGTGGGT TCAG

4.8. Variant identification

QIAamp DNA Mini Kit (Qiagen) was used according to manufactures guidelines for DNA isolation. PCR products were sequenced by Sanger sequencing with primers listed in 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.101855.

References

Hahn, A.F., Boman, H., 2016. Cold-Induced Sweating Syndrome including Crisponi Syndrome. GeneReviews Seattle, WA Univ Washington, pp. 1993–2018.
Buers, I., Pennekamp, P., Nitschke, Y., Lowe, C., Skryabin, B.V., Rutsch, F., 2016. Lmbrd1 expression is essential for the initiation of gastrulation. J. Cell. Mol. Med. 20, 1523–1533.
Barch, M.J., Knutsen, T., Spurbeck, J.L. (Eds.), 1997. The AGT Cytogenetics Laboratory Manual. Lippincott Williams & Wilkins.
Hecker, K.H., Roux, K.H., 1996. High and low annealing temperatures increase both specificity and yield in touchdown and stepdown PCR. Biotechniques 20, 478–485.
Akane, A., Shiono, H., Matsubara, K., Nakahori, Y., Seki, S., Nagafuchi, S., Yamada, M., Nakagome, Y., 1991. Sex identification of forensic specimens by polymerase chain reaction (PCR): two alternative methods. Forensic. Sci. Int. 49, 81–88.