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Lab Resource: Multiple Cell Lines

Generation of induced pluripotent stem cell (iPSC) lines carrying a heterozygous (UKWMPi002-A-1) and null mutant knockout (UKWMPi002-A-2) of Cadherin 13 associated with neurodevelopmental disorders using CRISPR/Cas9

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A B S T R A C T

Fibroblasts isolated from a skin biopsy of a healthy 46-year-old female were infected with Sendai virus containing the Yamanaka factors to produce transgene-free human induced pluripotent stem cells (iPSCs). CRISPR/Cas9 was used to generate isogenic cell lines with a gene dose-dependent deficiency of CDH13, a risk gene associated with neurodevelopmental and psychiatric disorders. Thereby, a heterozygous *CDH13* knockout ($CDH13^{+/-}$) and a *CDH13* null mutant ($CDH13^{-/-}$) iPSC line was obtained. All three lines showed expression of pluripotency-associated markers, the ability to differentiate into cells of the three germ layers *in vitro*, and a normal female karyotype.

Resource table

Unique stem cell lines identifier	1. UKWMPi002-A 2. UKWMPi002-A-1 3. UKWMPi002-A-2
Alternative names of stem cell lines	$CDH13^{+/+}$ (UKWMPi002-A) $CDH13^{+/-}$ (UKWMPi002-A-1) $CDH13^{-/-}$ (UKWMPi002-A-2)
Institution	Division of Molecular Psychiatry, Center of Mental Health, University Hospital Würzburg, Germany
Contact information of distributor	Klaus-Peter Lesch, kplesch@mail.uni-wuerzburg.de
Type of cell lines	iPSC
Origin	Human, Age: 46, Sex: female
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrative Sendai virus
Multiline rationale	Isogenic *Cadherin-13* (*CDH13*)-edited cell lines
Gene modification	Yes
Type of modification	Induced insertion/deletion mutation
Associated disease	Neurodevelopmental and psychiatric disorders, e.g. autism-spectrum disorders (ASD), attention-deficit/hyperactive disorder (ADHD), depression
Gene/locus	

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(*continued*)

	CDH13; Chr16q23.3; Entrez Gene ID1012 *CDH13*$^{+/+}$: NG_052819.1 *CDH13*$^{+/-}$: NM_001257.5: c. [=]; [13delA] *CDH13*$^{-/-}$: NM_001257.5: c.[22_25delGTTC]; [22_23insG]
Method of modification	CRISPR/Cas9
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	N/A
Cell line repository/bank	N/A
Ethical approval	Ethics Committee, Medical Faculty, University Hospital Würzburg, 96/11

1. Resource utility

Copy-number and single-nucleotide variation in *Cadherin-13* (*CDH13*), encoding a cell adhesion molecule involved in brain development and plasticity, is associated with neurodevelopmental and psychiatric disorders. Isogenic induced pluripotent stem cell (iPSC) lines with a gene dose-dependent deficiency of *CDH13* will facilitate investigation of CDH13 in cellular processes, neuronal function, and organoid network activity.

2. Resource details

Cadherin-13 (CDH13) is a cell adhesion molecule which regulates a wide range of cellular processes in brain development and plasticity (Forero et al., 2020; Rivero et al., 2015). Genetic variation in *CDH13* is associated with neurodevelopmental and psychiatric disorders in numerous genome-wide association, copy-number variant, and whole-exome sequencing studies. Rare deletions at the *CDH13* locus are linked to autism spectrum disorders (Sanders et al., 2015) indicating clinical relevance of loss-of-function mutations. Moreover, numerous studies reported associations of *CDH13* variants with attention-deficit/hyperactivity disorder, substance use/dependence, and depression (Howard et al., 2019).

We therefore developed a corresponding human iPSC-based *in vitro* model. In brief, dermal fibroblasts were isolated from a 46-year-old healthy female and reprogrammed into iPSCs using the CytoTune-iPS Reprogramming Kit 2.0 (Thermo Fisher). iPSC-like colonies were manually picked and expanded under feeder-free conditions using StemMACS™ iPS-Brew XF (Miltenyi Biotec). From a sub-clone, we generated two isogenic cell lines using the CRISPR/Cas9 system (Ran et al., 2013). To generate *CDH13* knockout (KO) lines, we designed sgRNAs targeting the start of the open reading frame Fig. 1A through which insertion or deletion of nucleotides result in a frameshift of CDH13 translation. After additional single cell expansion of the CRISPRed clones, we identified a heterozygous *CDH13* KO (*CDH13*$^{+/-}$) and a *CDH13* null mutant (*CDH13*$^{-/-}$) iPSC line. PCR Cloning (NEB) and subsequent DNA sequencing (LGC genomics) revealed the introduced mutations on both alleles. The *CDH13*$^{+/-}$ line carries an unmodified, wild type allele and a deletion of one nucleotide on the other allele (Fig. 1B). The *CDH13*$^{-/-}$ line carries one allele with a deletion of four nucleotides and the second allele with an insertion of one nucleotide (Fig. 1C). We differentiated the iPSCs into their mesodermal state to confirm the modifications of *CDH13* at the protein level (Fig. 1DD). Additionally, the null mutant was electro-physiologically confirmed in differentiated neurons by N. Nadif Kasri (manuscript in preparation).

All three iPSC lines displayed typical pluripotent stem cell morphology (Fig. 1E). To prove the pluripotent differentiation potential of the three iPSC lines, EBs were generated and cultured in suspension in

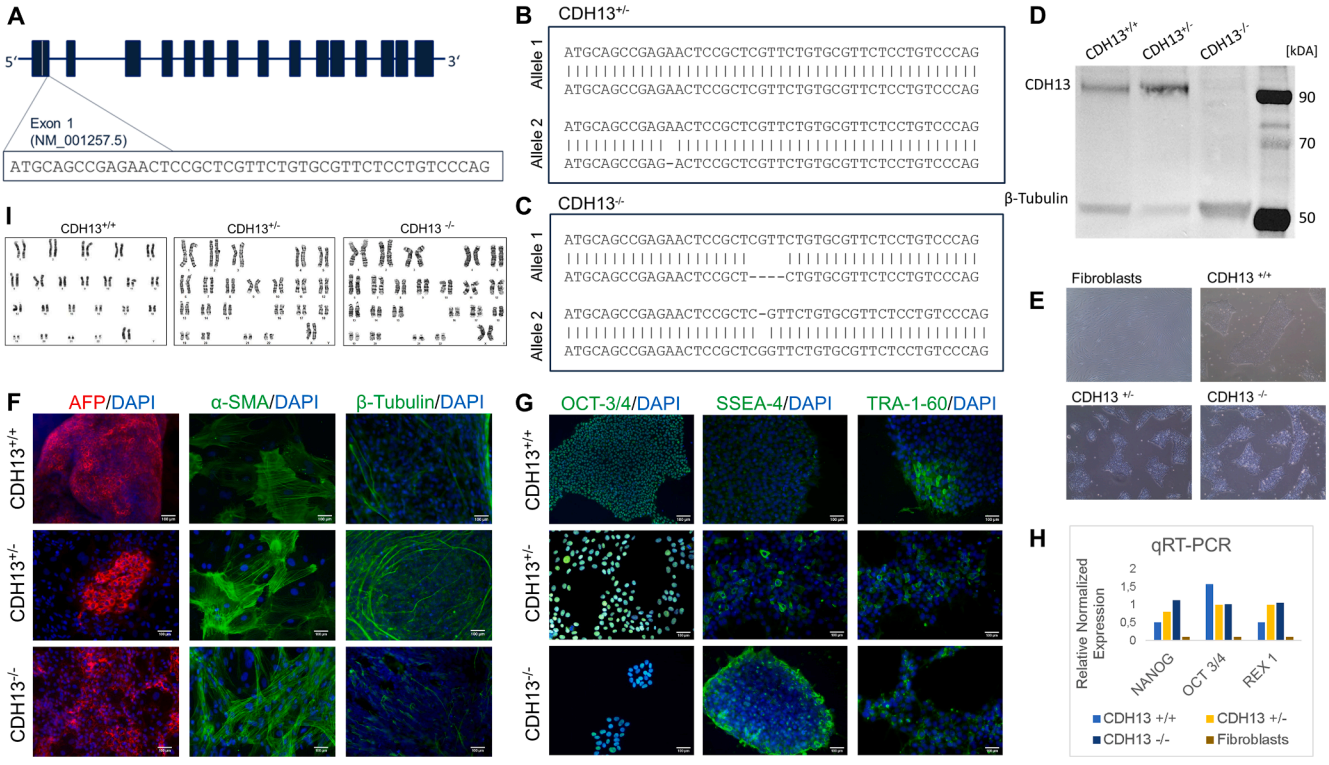

Fig. 1. Characterization of isogenic iPSC lines (A–I). **A** Start of the open reading frame NM_001257.5. B *CDH13*$^{+/-}$: NM_001257.5: c [=]; [13delA]. **C** *CDH13*$^{-/-}$: NM_001257.5: c.[22_25delGTTC]; [22_23insG]. **D** *CDH13* at the protein level. **E** Generation of iPSCs from patient's skin fibroblasts. **F** IPSCs were differentiated into cells of all three germ layers (Mesoderm: α-SMA; Endoderm: AFP; Ectoderm: β-Tubulin; nuclei stained with DAPI). **G** Immunostaining of pluripotency markers TRA-1-60, SSEA-4 and OCT-3/4, nuclei stained with DAPI. **H** Pluripotency quantitavely determined by qRT-PCR (NANOG, OCT 3/4, REX 1). **I** Standard G-banding revealed no numerical or structural chromosome abnormalities.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UKWMPi002-A	*CDH13*$^{+/+}$	Female	46	Caucasian	CDH13 NG_052819.1	Healthy
UKWMPi002-A-1	*CDH13*$^{+/-}$	Female	46	Caucasian	CDH13 NM_001257.5: c. [=]; [13delA]	Associated with neurodevelopmental and psychiatric disorders
UKWMPi002-A-2	*CDH13*$^{-/-}$	Female	46	Caucasian	CDH13 NM_001257.5: c.[22_25delGTTC]; [22_23insG]	Associated with neurodevelopmental and psychiatric disorders

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Light microscopy	hESC-like morphology (compact, dense, roundly shaped colonies with sharp edges)	Fig. 1E
Phenotype	Qualitative analysis (Immunocytochemistry)	Expression of pluripotency-associated markers TRA-1–60, SSEA-4 and OCT-3/4: positive	Fig. 1G
	Quantitative analysis (RT-qPCR)	NANOG, OCT3/4, REX1: positive	Fig. 1H
Genotype	Karyotype (G-banding) and resolution	46, XX; Resolution 450–550	Fig. 1I
Identity	STR analysis	DNA Profiling: performed	Submitted in archive with journal
		D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, AMEL, D5S818, FGA, D19S433, vWA, TPOX and D18S51. All three generated iPSC lines and fibroblast cell line matched	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	DNA Sequencing	*CDH13*$^{+/+}$ NG_052819.1 *CDH13*$^{+/-}$ NM_001257.5: c. [=]; [13delA] *CDH13*$^{-/-}$ NM_001257.5: c.[22_25delGTTC]; [22_23insG]	Fig. 1B Fig. 1C
	DNA Sequencing	Most likely off-target (gRNA mismatches): NM_001146274 NM_004853.3 NM_001040000.3 NM_207116 NM_001369490 NM_003800.5: All checked potential off-targets revealed unmodified	Fig. 1A
Microbiology and virology	Mycoplasma	LookOut® Mycoplasma PCR Detection Kit: negative	Supplementary Fig. S1C
Differentiation potential	Embryoid body formation and undirected differentiation	Expression of α-smooth muscle actin, α-feto protein and β-Tubulin III: positive	Fig. 1F
Donor screening (OPTIONAL)	HIV 1, Hepatitis B, Hepatitis C	negative	Submitted in archive with journal
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

serum-containing medium for seven days and terminally differentiated in adherent culture conditions for up to three weeks. All three iPSC lines expressed the following germ layer markers: α-SMA (mesoderm), AFP (endoderm) and β-Tubulin (ectoderm) (Fig. 1F). All iPSC lines were investigated for the expression of pluripotency markers via immunofluorescence (OCT 3/4, SSEA-4, TRA-1–60) (Fig. 1G) and quantitatively determined by qRT-PCR (NANOG, OCT 3/4, REX 1) Figure Supplementary 1: **A** Potential off-target sites for both sgRNAs. **B** The absence of Sendai virus specific transcripts was confirmed by RT-PCR. **C** Negative mycoplasma test. (Fig. 1H). Standard G-banding revealed no numerical or structural chromosome abnormalities (Fig. S1). The absence of Sendai virus specific transcripts was confirmed by RT-PCR (Fig. S1B). Short tandem repeat (STR) analysis showed that parental fibroblasts and newly created iPSCs shared alleles with a 100% match. All iPSC lines were mycoplasma-negative (Fig. S1C). Taken together, we generated three iPSC lines which showed expression of pluripotency markers, the capacity to differentiate into all the germ layers, and a normal karyotype (Tables 1 and 2). Thus, these lines are well suited to study the effect of human CDH13 deficiency in cellular processes and therefore to elucidate the role of CDH13 in the etiopathogenesis of a wide spectrum of neurodevelopmental and psychiatric disorders.

3. Materials and methods

3.1. Skin biopsy preparation

Human dermal fibroblasts were isolated from a healthy individual and cultured in fibroblast growth medium (DMEM, 10% fetal bovine serum (FBS), 1 μg/ml Gentamicin).

3.2. Reprogramming of fibroblasts into iPSCs

Fibroblasts were reprogrammed using CytoTune-iPS Reprogramming Kit 2.0 (Thermo Fisher). Fibroblasts were transduced with Sendai virus for 24 h. After seven days, cells were seeded on irradiated mouse embryonic fibroblasts in reprogramming medium. Colonies with hESC-like appearance were manually transferred to Matrigel™ (Corning) coated 6-well plates in StemMACS™iPS-BrewXF (Miltenyi Biotec) supplemented with 10 μM Y27632 (Miltenyi Biotech) only on the first day. Medium was changed every day.

3.3. CRISPR/Cas9

The sgRNAs were designed with the software tool ‘Design CRISPR

Table 3
Reagents details.

Antibodies used for immunocytochemistry/western blot			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-OCT-3/4	1:50	Santa Cruz, Cat# sc5279, RRID:AB_628051
	Mouse anti-TRA-1–60	1:50	Santa Cruz, Cat# sc21705, RRID: AB_628385
	Mouse anti-SSEA-4	1:200	Thermo Fisher, Cat# MA1-021, RRID: AB_2536687
Differentiation markers	Mouse anti-betaTubIII	1:1000	Promega, Cat# G7121, RRID:AB_430874
	Rabbit anti-AFP	1:400	Dako, Cat# A0008, RRID:AB_2650473
	Mouse anti-α-SMA	1:1000	R&D Cat# MAB1420, RRID:AB_262054
Secondary antibodies	Goat Anti-Mouse555	1:400	Thermo Fisher, Cat#A-21422, RRID: AB_2535844
	Goat Anti-Rabbit555	1:400	Thermo Fisher, Cat# A11034, RRID: AB_2576217
	Goat Anti-Mouse488	1:400	Thermo Fisher, Cat# A11029, RRID: AB_138404
CDH13	Goat Anti-Human Cadherin-13	1:200	R&D Systems: Cat# AF3264
β-Tubulin	Anti-βIII Tubulin mAb	1:1000	Promega: Cat# G7121; RRID:AB_430874
Secondary antibodies	IRDye® 680RD Donkey anti Goat IgG anti-IgG	1:5000	LI-COR Biosciences: Cat#:926-68074 RRID:AB_2650427; AB_10956736
	IRDye® 680RD Donkey anti-Mouse IgG	1:10000	LI-COR Biosciences: Cat#: 926-68072 RRID: AB_2814912; AB_10953628

Primers		
	Target	Forward/Reverse primer (5′–3′)
Sendai virus detection	iPSC-SeV	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
	iPSC-KOS	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
	iPSC-Klf4	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA
	iPSC-c-Myc	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGAT
Pluripotency markers (qPCR)	REX1	AGGTGGCATTGGAAATAGCAGA/ AGTGGGGTGGGTTTGCCTA
	Oct3/4	CCCACACTGCAGCAGATCA/ TGTGCATAGTCGCTGCTTGA
	NanoG	CTGAGATGCCTCACACGGAG/ TGTTTGCCTTTGGGACTGGT
House-Keeping Genes (qPCR)	GAPDH	CTCATGACCACAGTCCATGCC/ GCCATCCACAGTCTTCTGGGT
	HPRT1	CTTGGTCAGGCAGTATAATCCAA/ CTTATAQTCCAACACTTCGTGGGG
	RS27A	GGTTAAGCTGGCTGTCCTGAA/ AGAAGGGCACTCTCGACGAA
	UBC	Qiagen
	ACTB	Qiagen
Targeted mutation analysis/ sequencing	CDH13 exon1	CATTGCCCAGCGTGATTTGTGAG/ TCCCTACCGAGCCCCGATCTG
sgRNA #1 CDH13$^{+/-}$	CDH13 exon1	GCACAGAACGAGCGGAGTTCT/ AGAACTCCGCTCGTTCTGTGC
	NM_001146274	TTCTGCTGTGAGTGGTTTTGA/ ACTTCCAACCCAAACTGACCC

Table 3 (*continued*)

Primers		
	Target	Forward/Reverse primer (5′–3′)
sgRNA #1 off-target PCR/ sequencing	NM_004853.3	GCCGGAGTAGAGTTACAGCC/ CCTGGGCATCCTGAGACTTG
	NM_001040000.3	GGGGAACTGTCAGCCATAGA/ CTAATTGCCCCCTGTCACCC
sgRNA #2 CDH13$^{-/-}$	CDH13 exon1	GAGGAGAACGCACAGAACGAG/ CTCGTTCTGTGCGTTCTCCTC
sgRNA #2 off-target PCR/ sequencing	NM_207116	TCTGCTGACTCACCTCCTCA/ GCTGTTTTGATGGGAACGGG
	NM_001369490	GAGGTCAGAGAATGTGCACCA/ GTCAGGATTCAGGGCCAGTG
	NM_003800.5	GGTGGTGTTATTGGTTTAAG/ CTTATAGTCCCTAGGCTAAG

Guides' from Benchling (https://www.benchling.com/) (Table 3). SgRNAs were cloned into the pSpCas9(BB)-2A-Puro (PX459) V2.0 plasmid (Ran et al., 2013) (Addgene: #62988). Nucleofection of 800,000 iPSCs with 5 μg plasmid was performed by using the Nucleofector™ System (LONZA). Puromycin (0.7 μg/ml; Invivogen) selection was applied one day after for 48 h. After three weeks, surviving colonies were selected and individually transferred into pre-coated wells. For protein isolation, the iPSCs were differentiated into mesodermal tissue using the StemMacs™ Trilineage Differentiation Kit (Miltenyi Biotec).

3.4. Western blot

20 μg protein was loaded on a polyacrylamide gel (NuPAGE™ Novex™, Invitrogen™). Proteins were transferred onto a nitrocellulose membrane and stained with antibodies (Table 3). Infrared fluorescence was visualized with the Fusion FX system.

3.5. Genotyping and sequencing

To identify mutations and potential CRISPR/Cas9 off-target effects, isolated DNA was amplified by PCR (Table 3). PCR Products were purified with the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel) and sequenced (LGC Genomics). Biallelic modifications were verified using the PCR Cloning Kit (NEB). SnapGeneViewer (version: 4.2.6) was used to detect genomic alterations.

3.6. Karyotype analysis

Karyotypes were verified by GTG-banding analysis and carried out by Creative Biorray using 20 metaphases for CDH13$^{+/+}$. For CDH13$^{+/-}$ and CDH13$^{-/-}$, the analysis was carried out by the Institute of Human Genetics, University of Würzburg, using 10 metaphases. Samples were examined with a resolution of 450–500 bands using an Axioskop (Zeiss) microscope.

3.7. Germ layer differentiation

IPSCs were seeded on ultra-low attachment plates (Corning) in differentiation medium (DMEM, 10% FBS, 1% NEA, 100 μM β-mercaptoethanol) with 10 μM Y27632. After seven days in suspension, EBs were plated on 0.1% gelatine (PAN Biotech)-coated plates for undirected differentiation and specific germ layer markers were tested by immunofluorescence three weeks later.

3.8. Immunofluorescence analysis

Cells were fixed with 4% paraformaldehyde (Roth), blocked (5% FBS, 1% BSA in PBS) and permeabilized with 0.2% Triton X-100 (intracellular markers; Sigma) for 30 min at RT. Primary and secondary antibodies (Table 2), and DAPI (300 nM; Sigma) were incubated for 12 h

at 4 °C and 1 h at room temperature, respectively. Cells were visualized under an inverted fluorescence microscope (Olympus).

3.9. STR analysis

STR analysis was conducted by Eurofins Genomics. DNA isolation was performed using the cell pellet (cell layer). Genetic characteristics were determined by PCR single-locus technology.

3.10. Mycoplasma contamination detection

The absence of mycoplasma contamination was confirmed using LookOut® Mycoplasma PCR Detection Kit (Sigma-Aldrich).

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.

Acknowledgments

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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.2021.102169.

References

Forero, A., Ku, H.P., Belen Malpartida, A., Wäldchen, S., Alhama-Riba, J., Aboagye, B., Ding, Y.Q., Blum, R., Sauer, M., Rivero, O., Lesch, K.P., 2020. Serotonin (5-HT) neuron-specific inactivation of Cadherin-13 impacts 5-HT system formation and cognitive function. Neuropharmacol. 168, 108018. https://doi.org/10.1016/j.neuropharm.2020.108018.

Rivero, O., Selten, M.M., Sich, S., Popp, S., Bacmeister, L., Amendola, E., et al., 2015. Cadherin-13, a risk gene for ADHD and comorbid disorders, impacts GABAergic function in hippocampus and cognition. Transl. Psychiatry 5, Pe655. https://doi.org/10.1038/tp.2015.152.

Sanders, S.J., He, X., Willsey, A.J., Ercan-Sencicek, A.G., Samocha, K.E., Cicek, A.E., et al., 2015. Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. Neuron 87, 1215–1233. https://doi.org/10.1016/j.neuron.2015.09.016.

Howard, D.M., Adams, M.J., Clarke, T.K., et al., 2019. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. Nat. Neurosci. 22, 343–352. https://doi.org/10.1038/s41593-018-0326-7.

Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8, 2281–2308. https://doi.org/10.1038/nprot.2013.143.