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Generation of an iPSC line (UNINAi001-A) from a girl with neonatal-onset epilepsy and non-syndromic intellectual disability carrying the homozygous KCNQ3 p.PHE534ILEfs*15 variant and of an iPSC line (UNINAi002-A) from a non-carrier, unaffected brother

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A B S T R A C T

Heterozygous variants in the KCNQ3 gene cause epileptic and/or developmental disorders of varying severity. Here we describe the generation of induced pluripotent stem cells (iPSCs) from a 9-year-old girl with pharmacodependent neonatal-onset epilepsy and intellectual disability who carry a homozygous single-base duplication in exon 12 of KCNQ3 (NM_004519.3: KCNQ3 c.1599dup; KCNQ3 p.PHE534ILEfs*15), and from a non-carrier brother of the proband. For iPSC generation, non-integrating episomal plasmid vectors were used to transfect fibroblasts isolated from skin biopsies. The obtained iPSC lines had a normal karyotype, showed embryonic stem cell-like morphology, expressed pluripotency markers, and possessed trilineage differentiation potential.

Resource Table

Unique stem cell lines identifier	UNINAi001-A; UNINAi002-A
Alternative names of stem cell lines	Q3HOMOFS1 (UNINAI001-A); Q3CONTR (UNINAI002-A)
Institution	Division of Pharmacology, Department of Neuroscience, University of Naples "Federico II", Via Pansini 5, 80,131 Naples, Italy
Contact information of distributor	Taglialatela Maurizio; maurizio.taglialatela@unina.it
Type of cell lines	iPSC
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Nucleofection of non-integrative, episomal reprogramming plasmids encoding for OCT4, SOX2, L-MYC, KLF4, and Lin28
Multiline rationale	Control and disease pair from the same family
Gene modification	YES
Type of modification	Congenital
Associated disease	Epilepsy with non-syndromic intellectual disability
Gene/locus	KCNQ3 (chr8:g133150233dup in GRCh37, NM 004519.3:c.1599dup (p.Phe534llefs*15) in homozygosity
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	2/2021
Cell line repository/bank	Human Pluripotent Stem Cell Registry (hPSCreg)
Ethical approval	This study was approved by the institutional review board (n°DC2011-1332) of the Université de Bourgogne in Dijon (FR).

1. Resource utility

iPSC lines generated from patients carrying pathogenic variants in KCNQ3 offers a suitable cellular model to dissect pathological

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mechanisms underlying neonatal-onset epilepsies and developmental disabilities, and a platform for new antiepileptic drugs screening. The iPSC line from a non-affected non-carrier brother represents a familial control with minimal genetic differences.

2. Resource details

Heterozygous pathogenic variants in *KCNQ3*, encoding for Kv7.3 voltage-dependent K+ channel subunits, have been found in families affected with benign familial neonatal seizures (BFNS), an autosomal-dominantly inherited form of epilepsy characterized by multifocal seizures beginning in the first days of life, with generally normal interictal electroencephalograms (EEGs) and subsequent neuropsychological development (Charlier et al., 1998). Although BFNS is mostly a self-limiting disease and seizures disappear spontaneously after few weeks or months, some individuals in BFNS pedigrees show more severe phenotypes with persistent seizures and some degree of intellectual disability (ID); de novo pathogenic variants are also found in sporadic cases of children with developmental and/or epileptic encephalopathies (DEEs), ID apparently without epilepsy, cortical visual impairment and ID, and autism spectrum disorder (Nappi et al., 2020). In both inherited or de novo cases of KCNQ3-related disorders, pathogenic variants are missense and occur in heterozygosity (Sands et al., 2019) or in compound heterozygosity (Ambrosino et al., 2018).

We recently described a 9-year-old girl with pharmacodependent neonatal-onset epilepsy and non-syndromic ID carrying a homozygous single base duplication in *KCNQ3*, which reduced transcript and protein abundance, and impeded channel function (Lauritano et al., 2019). The parents and one of the two brothers of the proband were heterozygous carriers for the KCNQ3 variant, whereas the eldest brother carried two copies of the wild-type allele; all these individuals had normal psychomotor and cognitive development with no history of seizures.

iPSCs were generated from primary dermal fibroblasts at passage 5 obtained from the proband (previously referred to as individual II-3) and the unaffected, non-carrier brother (previously referred to as individual II-1) (Lauritano et al., 2019). Characterization of these iPSCs (UNINAi001-A: proband; UNINAi002-A: control brother; Table 1) was performed after manual picking and expansion in feeder free condition of alkaline phosphatase positive clones. The iPSC colonies displayed round shaped stem cells morphology with sharp borders. Within colonies, cells were tightly-packed, small and with a large nucleus/cytoplasm ratio (Fig. 1A). The stem cell properties of the clones were confirmed by positive immunostaining with the pluripotency markers SSEA4 and NANOG (Fig. 1B). RT-PCR also revealed abundant expression of the endogenous pluripotency genes *OCT4 and NANOG*, whereas expression of plasmids encoding for the exogenous episomal reprogramming factors was undetectable (Fig. 1C).

G-banding showed that cells within the clones were genetically stable and had a normal karyotype (Fig. 1D; left panel: UNINAi002-A, control individual; right panel: UNINAi001-A, proband). Sanger sequencing confirmed the respective genotype in each clone from the two individuals (Fig. 1E). Short tandem repeats (STR) analysis demonstrated an identical DNA profile at 24 polymorphic loci with the parental fibroblasts (data submitted in the Journal's archive). Pluripotency of the selected iPSC clones was further validated by differentiating iPSCs into all three embryonic germ layers. Immunoreactivity for NESTIN (ectoderm), smooth muscle actin (SMA; mesoderm), and SRY-related HMG-box17 (SOX17; endoderm) was observed (Fig. 1F). All three iPSC lines also tested negative for mycoplasma (data not shown) (Table 2).

3. Materials and methods

3.1. Fibroblasts culturing and reprogramming

Dermal fibroblasts were obtained via 4 mm^2 punch skin biopsies performed at the Dijon University Hospital. Fibroblasts were cultivated at 37 °C in Dulbecco's Modified Eagle Media (DMEM; Gibco, Thermo Fisher, Waltham, MA, USA) supplemented with 10% FCS. Fibroblasts reprogramming was carried out by Epi5 Episomal iPSC reprogramming kit (Invitrogen), in feeder-free conditions. Vectors were delivered into $1x10^7$ fibroblasts via nucleofection using the Neon Transfection System (Invitrogen). Emerging iPSC colonies were manually picked, transferred on GelTrex (LDEV free hESC-qualified, Life Technologies) -coated plates and cultured in Essential-8 medium (E8, Life Technologies).

3.2. Immunofluorescence

Alkaline Phosphatase Live Stain kit (Life Technologies) was used to detect and select emerging iPSC colonies. For immunofluorescence staining, iPSCs were fixed with 4% paraformaldehyde, permeabilized with 0.1% Tween-20 for 5 min, blocked with 0.5% BSA for 1 h at RT and incubated overnight at 4 °C with primary antibodies followed by incubation with secondary antibodies. Nuclei were visualized using Hoechst 33258. Coverslips were mounted in Fluoromount G (eBioscience, Hatfield, Hertfordshire, UK); images were acquired with a Zeiss inverted LSM 700 confocal laser scanning microscope (Carl Zeiss, Jena, Germany) and analyzed with ImageJ (NIH, Bethesda, MD, USA).

3.3. Mycoplasma detection

hiPSC lines were tested for mycoplasma contamination by Mycoplasma PCR Detection Kit (ABM Inc.) according to manufacturer's protocol.

3.4. In vitro differentiation protocol

To test pluripotency, neuroectodermal differentiation was initiated by plating iPSC into PSC Neural Induction Medium (Life Technologies). At day 7 of neural induction, cells were dissociated with Accutase (Life Technologies) and plated on Geltrex-coated dishes in NSC expansion medium (Gibco). For mesodermal differentiation experiments, iPSCs were dissociated, plated on Geltrex-treated plates, and cultured in E8 medium for about 48 h at 37 °C, 5% CO_2. When cells reached 30–50% confluency, E8 medium was replaced with Cardiomyocyte Differentiation Medium A (Gibco) and cultured for 48 h. At day 3 of differentiation cells were shifted to Cardiomyocyte Differentiation Medium B (Gibco) for 48 h, and, ultimately, in Cardiomyocyte Maintenance Medium A (Gibco) for an additional week. For endodermal differentiation, iPSC were cultured in E8 essential medium (Gibco) on Vitronectin-coated plates (Gibco) at a density of 0.01–0.04 × 10^6 cells/cm^2 overnight at 37 °C, 5% CO_2. After 24 h, the medium was replaced with Definitive

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UNINAi002-A	*Control*	*Male*	*15*	*Arab (Morocco)*	*KCNQ3 (*NM 004519.3; *WT)*	*None (unaffected, non-carrier brother of the proband)*
UNINAi001-A	*Proband*	*Female*	*9*	*Arab (Morocco)*	KCNQ3 (NM 004519.3; homozygous for the c.1599dup (p. Phe534Ilefs*15) variant)	*Epilepsy and ID*

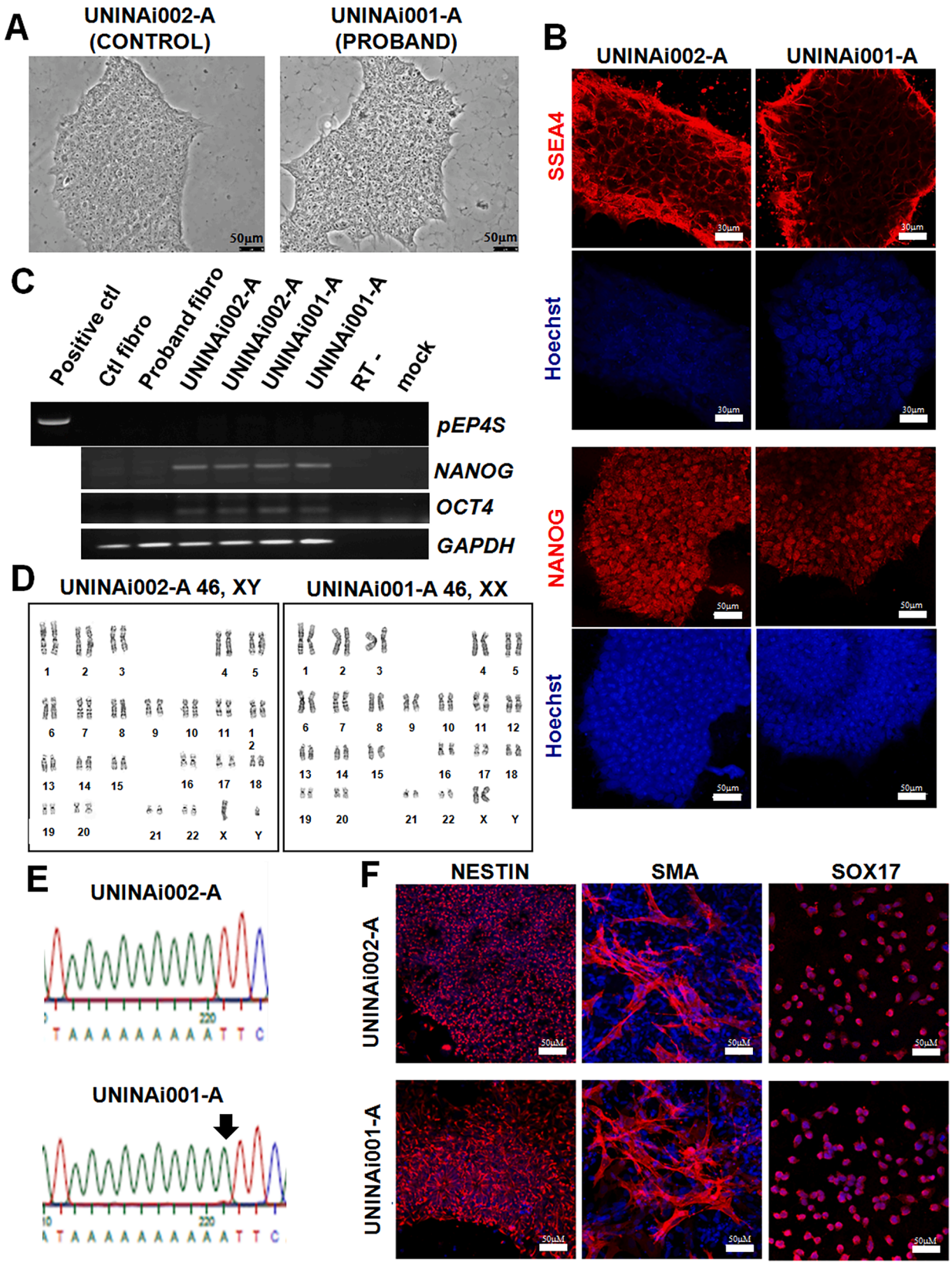

Fig. 1. hiPSC characterization: morphology (A), pluripotency (B, C), karyotype (D), DNA sequences (E), and differentiation potential (F).

Endoderm (DE) induction medium A (Gibco) and the cells were cultured for additional 24 h. At day 2 of induction, DE induction medium A was removed and cells cultured for further 24 h into DE induction medium B.

3.5. RNA extraction and RT-PCR

Total RNA was extracted using the TriReagent (Sigma, St. Louis, MO, USA). 1 µg of total RNA was retrotranscribed with the High Capacity

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Stem cell-like morphology	Fig. 1 panel A
Phenotype	Qualitative analysis: Immunocytochemistry	Positive staining for the pluripotency markers NANOG and SSEA4	Fig. 1 panel B
	Quantitative analysis: PCR	Expression of NANOG and OCT4 endogenous transcripts; negative for exogenous episomal reprogramming vectors	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46 XY (UNINAi002-A); 46 XX (UNINAi001-A). 400-band resolution	Fig. 1 panel D
Identity	STR analysis	Tested 22 STR loci (D3S1358; vWA; D16S539; CSF1PO; TPOX; D8S1179; D21S11; D18S51; DYS391; D2S441; D19S433; TH01; FGA; D22S1045; D5S818; D13S317; D7S820; SE33; D10S1248; D1S11656; D12S391; D2S1338) and 2 sex-determining markers (Y indel and Amelogenin).	Submitted in archive with Journal
Mutation analysis	Sequencing	Confirmation of the KCNQ3 c.1599dup in the proband clone; no KCNQ3 mutation detected in the control brother clone	Fig. 1 panel E
Microbiology and virology	Mycoplasma	Mycoplasma testing by Mycoplasma PCR Detection Kit (ABM Inc.), Negative	Available with authors
Differentiation potential	Directed differentiation	Immunofluorescence positivity for NESTIN (neuroectoderm), SMA (mesoderm) and SOX17 (endoderm)	Fig. 1 panel F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
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 Marker	Rabbit anti-NANOG	1:400	Cell Signaling Technology Cat# 4903, RRID: AB_10559205
Pluripotency Marker	Mouse anti-SSEA	1:200	Cell Signaling Technology Cat# 4755, RRID: AB_1264259
Differentiation Marker	Mouse anti-NESTIN	1:200	ThermoFisher Cat#MA1-110, RRID: AB_2536821
Differentiation Marker	Mouse anti-SMA	1:300	Abcam Cat#Ab7817, RRID:AB_262054
Differentiation Marker	Rabbit anti-SOX17	1:50	Thermo Fisher Scientific Cat# PA5-72815, RRID: AB_2718669)
Secondary antibody	AlexaFluor 647 Donkey Anti-Mouse IgG	1:100	Thermo Fisher Scientific Cat# A-31571, RRID: AB_162542
Secondary antibody	AlexaFluor 555 Donkey Anti-Rabbit IgG	1:100	Thermo Fisher Scientific Cat# A-31572, RRID: AB_162543
Nuclear staining	Hoechst 33258	1:5000	Thermo Fisher Scientific Cat# H3569, RRID: AB_2651133

Primers		
	Target	Forward/Reverse primer (5′-3′)
Episomal Plasmids (PCR)	pEP4SF1-R1 Reprogramming Plasmids (Product size: 544 bp)	ATCGTCAAAGCTGCACACAG/ CCCAGGAGTCCCAGTAGTCA
Pluripotency Markers (PCR)	endoNANOG (Product size: 193 bp)	TTTGGAAGCTGCTGGGGAAG/ ATGGGAGGAGGGGAGAGGA
Pluripotency Markers (PCR)	endoOCT4 (Product size: 103 bp)	AGTTTGTGCCAGGGTTTTTG/ ACTTCACCTTCCCTCCAACC
House-Keeping Gene (PCR)	GAPDH (Product size: 147 bp)	GTGGACCTGACCTGCCGTCT/ TGTCGCTGTGGGTGAGGAGG
Targeted mutation sequencing	Mutation KCNQ3 c.1599dup (product size: 510 bp)	CAATAAAGAGCGTTTCCGCAC/ TCCAGCTTCTTCCCCATGTC

cDNA RT Kit (Applied Biosystem, ThermoFisher Scientific, Milan, IT). To detect endogenous pluripotency genes and pEP4S reprogramming vectors, PCR was performed with the WonderTaq (Euroclone) and specific primers listed in Table 3.

3.6. Karyotype

Karyotyping was performed on iPSCs at passages 7–13 following standard cytogenetic procedures and according to the International System of Human Cytogenetic Nomenclature (ISCN) 2016. Briefly, cells were treated with 50 μl of colcemid solution (10 μg/ml in PBS) (MICROGEM) for 5 h at 37 °C, and then detached with trypsin/EDTA, and treated with hypotonic solution (KCl 0.56%) for 20 min at 37 °C and finally fixed in methanol/acetic acid solution (3:1 v/v). G-banding was performed on fixed metaphases at a resolution of 400 bands. Cells were incubated in SSC 2X solution for 3 h at 60 °C, washed with distilled water, and then with PBS 1X, treated with trypsin 1:250 (GIBCO) and stained with Giemsa's Reagent (Carlo Erba). Images of banded chromosomes were acquired on an Axio Imager Z1 microscope (Carl Zeiss, Milan, Italy) and karyotype analysis obtained from 16 metaphases using the IKAROS software imaging system (Metasystems, Altlussheim, Germany).

3.7. Sequencing

The genomic region encompassing the mutation was amplified using primers listed in Table 3 and the PCR products were sequenced (Eurofins Genomics, Milan, Italy).

3.8. Mycoplasma detection

Mycoplasma contamination was evaluated by PCR using the Myco-Sensor PCR Assay Kit (Agilent), following manufacturer's instructions.

3.9. STR analysis

Genomic DNA isolated by QIAamp DNA Mini Kit (Qiagen), was amplified for 22 STR loci and 2 sex-determining markers, Y indel and

Amelogenin, by GlobalFiler PCR Amplification Kit (Applied Biosystems) and analyzed on ABI-Prism 3500 genetic analyzer.

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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