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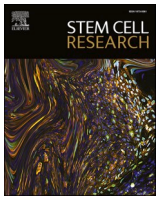

Lab Resource: Single Cell Line

Generation of an iPSC line from a patient with spastic paraplegia type 10 carrying a novel mutation in *KIF5A* gene

Serena Santangelo [a,b], Patrizia Bossolasco [b], Stefania Magri [c], Claudia Colombrita [b], Sabrina Invernizzi [b], Cinzia Gellera [c], Lorenzo Nanetti [c], Daniela Di Bella [c], Vincenzo Silani [b,d], Franco Taroni [c], Antonia Ratti [a,b,*]

[a] Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy
[b] Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy
[c] Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[d] Department of Pathophysiology and Transplantation, "Dino Ferrari" Center, Università degli Studi di Milano, Milan, Italy

A B S T R A C T

We generated an iPSC line from a patient with spastic paraplegia type 10 (SPG10) carrying the novel missense variant c.50G > A (p.R17Q) in the *N*-terminal motor domain of the kinesin family member 5A (*KIF5A*) gene.

This patient-derived *in vitro* cell model will help to investigate the role of different *KIF5A* mutations in inducing neurodegeneration in spastic paraplegia and in other *KIF5A*-related disorders, including Charcot-Marie-Tooth type 2 (CMT2) and amyotrophic lateral sclerosis (ALS).

Resource table

Unique stem cell line identifier	IAIi010-A
Alternative name(s) of stem cell line	KIF5A_C3
Institution	IRCCS Istituto Auxologico Italiano, Milan, Italy
Contact information of distributor	Antonia Ratti, antonia.ratti@unimi.it
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Ethnicity: Caucasian Age: 79 Sex: Female
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Autosomal dominant Spastic Paraplegia type 10 (SGP10)
Gene/locus	KIF5A, chromosome 12q13.13 NM_004984.3: c.50G > A (p.R17Q)
Date archived/stock date	October 2022
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/IAIi010-A
Ethical approval	Ethical committee Regione Lombardia, sezione Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy, Approval n.64

1. Resource utility

Allelic mutations in *KIF5A* gene are associated to different neurodegenerative disorders, such as spastic paraplegia type 10 (SPG10), axonal Charcot-Marie-Tooth type 2 (CMT2), and amyotrophic lateral sclerosis (ALS) as well as to neonatal intractable myoclonus (NEIMY) with distinct mutational hotspots.

We generated an iPSC line from a SPG10 individual carrying the novel missense mutation p.R17Q (c.50G > A) in KIF5A protein motor domain.

This iPSC line represents a new *in vitro* disease model to elucidate, upon differentiation into motoneurons, the pathomechanisms associated with *KIF5A* mutations.

* Corresponding author.
E-mail address: antonia.ratti@unimi.it (A. Ratti).

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2. Resource details

The kinesin family member 5A (*KIF5A*) gene encodes for the heavy chain of the kinesin motor protein involved in the anterograde transport of synaptic vesicles, RNA granules, mitochondria and neurofilaments along dendrites and axons in neurons.

KIF5A forms a dimer of identical heavy chains which associate with 2 identical 60–70-kDa light chains. The KIF5A heavy chain contains 3 domains: an *N*-terminal globular domain (motor domain), a long alpha-helical coiled coil domain (stalk domain), and a small C-terminal globular domain (cargo domain).

Interestingly, *KIF5A* missense mutations in the *N*-terminal motor domain have been linked to SPG10 and CMT2 (Reid et al., 2002), loss-of-function variants in the C-terminal cargo-binding domain to ALS (Nicolas et al., 2018; Brenner et al., 2018 Mar), while C-terminal variants with a dominant-negative effect to the neurodevelopmental disorder

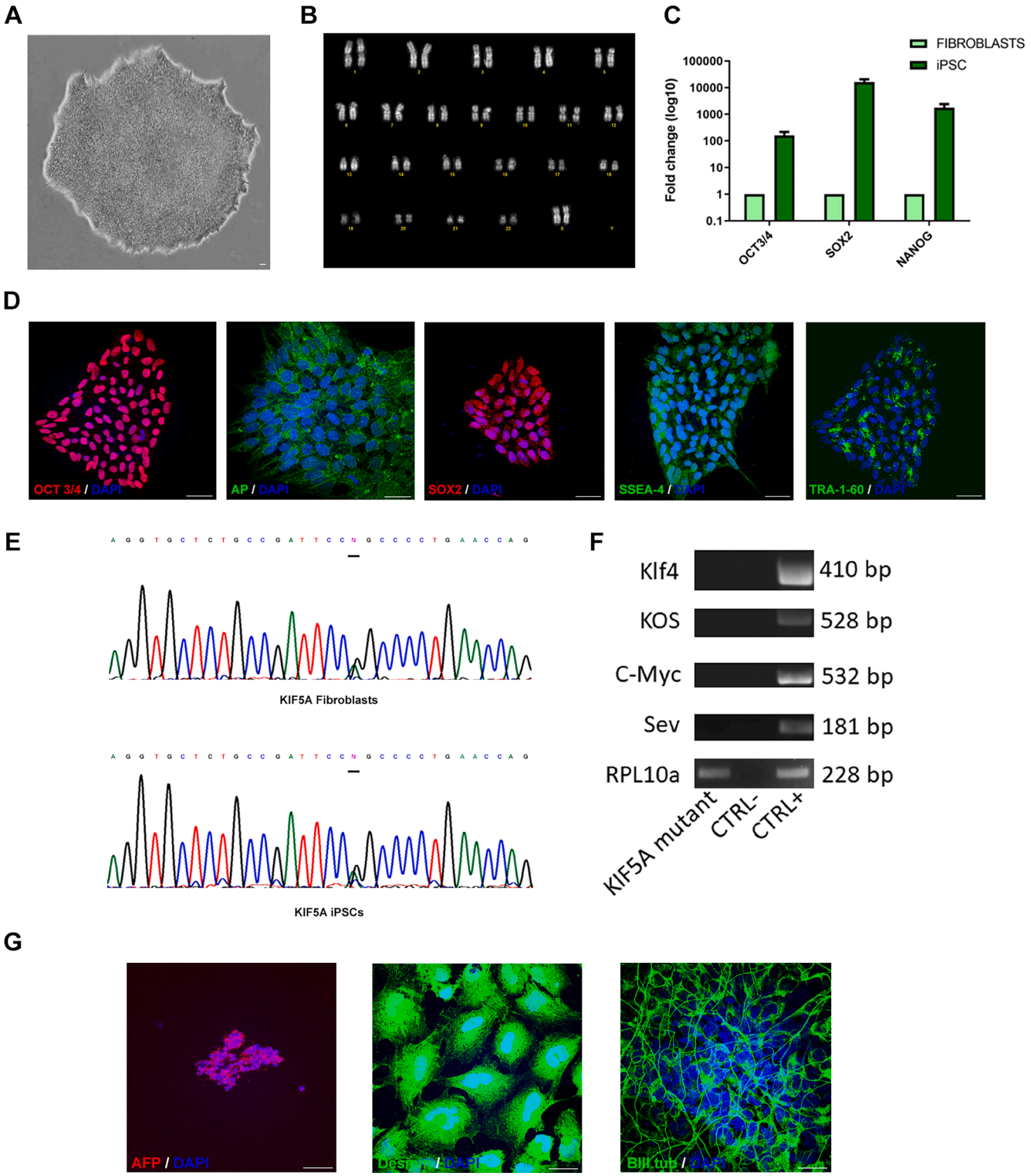

Fig. 1. Characterization of the generated iPSc line.

NEIMY (Duis et al., 2016).

The establishment of *in vitro* models to study *KIF5A*-related disorders is essential to understand the exact mechanisms whereby *KIF5A* allelic variants lead to neurodegeneration and cause different clinical phenotypes.

A targeted mutational screening of patients with hereditary spastic paraplegia allowed to identify a novel heterozygous missense variant (c.50G > A, p.R17Q) in the ATP-binding motor domain of *KIF5A* at the *N*-terminal region.

We isolated skin fibroblasts from the mutant SPG10 patient which were then reprogrammed into iPSC (IAIi010-A) using the Cytotune™-iPS 2.0 Sendai Reprogramming kit (ThermoFisher Scientific). The clonal iPSC colonies were isolated and, after expansion in E8 medium, they showed the typical stem cell morphology **(**Fig. 1**A)** and a normal karyotype (46, XX) at G-banding analysis **(**Fig. 1**B)**.

We evaluated their pluripotency status by the expression of OCT3/4, SOX2 and NANOG by Q-PCR (Fig. 1**C)** and of OCT3/4, AP (Alkaline Phosphatase), SOX2, SSEA-4 and TRA-1-60 pluripotency markers by immunofluorescence staining **(**Fig. 1**D)**.

Sanger sequencing confirmed the presence of the heterozygous c.50 G > A mutation in *KIF5A* exon 1 both in the SPG10 patient's fibroblasts and in the derivative iPSC line **(**Fig. 1**E).** Perfect matching with the original fibroblasts was also assessed by genotyping 22 different STRs (short tandem repeats) (data available on request).

Absence of the Sendai vector transcripts (Klf4, Kos, c-Myc, and Sev) was confirmed by semi-quantitative RT-PCR **(**Fig. 1**F).**

The ability of this iPSC line to spontaneously differentiate into derivatives of the three germ layers was assessed by *in vitro* embryoid body (EB) formation and immunofluorescence staining for specific markers: α-fetoprotein (AFP; endoderm), Desmin (mesoderm) and βIII-tubulin (ectoderm) **(**Fig. 1**G).**

The generated iPSC line tested negative for Mycoplasma infection **(Supplementary** Fig. 1**).**

The complete characterization is summarized in Table 1.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield image	Typical human pluripotent stem cell colony morphology	Fig. 1*A*
Phenotype	Qualitative analysis by immunocytochemistry	Expression of pluripotency markers: OCT3/4, Alkaline Phosphatase, SOX2, SSEA-4, TRA-1-60	Fig. 1*D*
	Quantitative analysis by Q-PCR	Expression of pluripotency markers: OCT3/4, SOX2, NANOG	Fig. 1*C*
Genotype	Karyotype (Q-banding) and resolution	46, XX	Fig. 1*B*
Identity	STR analysis	22 STR analyzed with 22 perfect matches	*Available from the Authors*
Mutation analysis	Sanger sequencing	Heterozygous mutation NM_004984.3: c.50G > A (p. R17Q) in *KIF5A*	Fig. 1*E*
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR: Negative	*Suppl.* Fig. 1
Differentiation potential	Embryoid body-derived germ layers	Expression of specific markers: endoderm (AFP), mesoderm (Desmin) and ectoderm (βIII-Tubulin)	Fig. 1*G*
List of recommended germ layer markers	Expression of specific germ layers markers by Immunofluorescence	Positivity for the AFP (endoderm), Desmin (mesoderm) and βIII-Tubulin (ectoderm) markers	Fig. 1*G*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

3. Materials and methods

3.1. Fibroblast reprogramming

Primary fibroblasts were obtained from skin biopsy and reprogramming was performed within the fifth passage *in vitro*. iPSCs were obtained using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) following manufacturer's instructions.

Briefly, 24 h after transduction, the fibroblast cell medium was replaced with fresh medium and cells were then cultured for 6 more days, replacing the medium every other day. At day 7, fibroblasts were plated in fresh fibroblast medium into 6-well plate pre-coated with hESC-Qualified Matrix (Corning). At day 8, the medium was replaced with Essential 8 medium (Thermo Fisher) until emerging colonies reached a suitable size to be manually picked. Colonies were grown at 37 °C, 5 % CO2 and splitted 1:10 using 0.5 mM EDTA solution.

3.2. Karyotyping

Standard cytogenetic procedures were used to analyse iPSC karyotype. Following overnight addition of Colcemid solution (KaryoMAX™, Thermo Fisher Scientific), chromosome analysis was achieved by Q-Band staining.

3.3. Immunocytochemistry

iPSCs cultured on glass coverslips were fixed in 4 % paraformaldehyde for 20 min and permeabilized with 0.3 % Triton X-100 (Sigma-Aldrich) for 10 min at room temperature. Cells were then incubated for 20 min in 10 % normal goat serum (NGS)(Gibco) in PBS and primary antibodies were added for 90 min at 37 °C.

After two washes with 10 % NGS/PBS, cells were stained with secondary antibodies. Nuclei were stained using DAPI (Sigma-Aldrich) and images acquired with a confocal microscope Nikon Eclipse Ti using a 60x oil-immersion objective. All the antibodies used are listed in Table 2.

3.4. In vitro spontaneous differentiation

Embryoid bodies (EBs) were generated by suspending iPSC in low-adhesion plates in HuES medium (DMEM/F12, NEAA, KSR, Glutamine, Pen/Strep, β-mercaptoethanol). After 7 days, EBs were plated on Matrigel-coated (Corning) coverslips and grown in Essential 8 medium for additional 10 days. Expression of the three-germ layer-specific markers was evaluated by immunofluorescence **(**Table 2**).**

3.5. Quantitative PCR (Q-PCR)

Total RNA was isolated with the TRIzol reagent (Invitrogen) and reversed transcribed using SuperScript II reverse transcriptase (Invitrogen). Q-PCR was performed using SYBRGreen reaction mix

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Mouse anti-Alkaline Phosphatase (AP)	1:250	Abcam cat# ab-108337	*RRID: AB_10862036*
	Mouse anti-SSEA-4	1:100	Invitrogen 14-8843-80	*RRID: AB_657847*
	Mouse anti-TRA-1-60	1:125	Invitrogen 14-8863-80	*RRID: AB_891612*
	Mouse anti-OCT-3/4	1:200	Santa Cruz Biotechnology cat#sc-5279	*RRID: AB_628051*
	Rabbit anti-SOX2	1:70	Abcam cat#ab15830	*RRID: AB_443255*
Differentiation Markers	Rabbit anti-βIII-Tubulin	1:500	Abcam cat#ab-52623	*RRID: AB_869991*
	Rabbit anti-Desmin	1:10	Chemicon Millipore cat#AB907	*RRID: AB_2092609*
	Mouse anti-Alpha-fetoprotein (AFP)	1:125	Invitrogen cat#14-6583-80	*RRID: AB_2865213*
e.g. Secondary antibodies	Alexa FluorTM 488 goat anti-mouse IgG (H + L)	1:500	Life Technologies cat# A-11001	*RRID: AB_2534069*
	Alexa FluorTM 555 goat anti-mouse IgG (H + L)	1:500	Life Technologies cat#A21422	*RRID: AB_2535844*
	Alexa FluorTM 555 goat anti-rabbit IgG (H + L)	1.500	Life Technologies cat# A-21428	*RRID: AB_2535849*
Nuclear stain	4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI)	2 μg/mL	Sigma-Aldrich D9542	
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qPCR)	OCT3/4	81 bp	Fwd: AGTGCCCGAAACCCACACTG Rev: CCACACTCGGACCACATCCT	
	SOX2	151 bp	Fwd: GGGAAATGGGAGGGGTGCAAAAGAGG Rev: CACCAATCCCATCCACACTCACGCAA	
	NANOG	154 bp	Fwd: TGAACCTCAGCTACAAACAG Rev: TGGTGGTAGGAAGAGTAAAG	
House-Keeping Genes (Q-PCR)	RPL10a	51 bp	Fwd: GAAGAAGGTGTTATGTCTGG Rev: TCTGTCATCTTCACGTGAC	
Sendai virus detection (RT-PCR)	Klf4	528 bp	Fwd: TTCCTGCATGCCAGAGGAGCCC Rev: AATGTATCGAAGGTGCTCAA	
	Kos	410 bp	Fwd: ATGCACCGCTACGACGTGAGCGC Rev: ACCTTGACAATCCTGATGTGG	
	c-Myc	532 bp	Fwd: TAACTGACTAGCAGGCTTGTCG Rev: TCCACATACAGTCCTGGATGATGATG	
	Sev	181 bp	Fwd: GGATCACTAGGTGATATCGAGC Rev: ACCAGACAAGAGTTTAAGAGATATGTATC	
	RPL10a	228 bp	Fwd: CAAGAAGCTGGCCAAGAAGTATG Rev: TCTGTCATCTTCACGTGAC	
Genotyping	22 STRs (CyberGene AB kit)	N/A	N/A	
Targeted mutation analysis/sequencing	KIF5A	370 bp	Fwd: CAGAGACTGAGCACCTGTCCTCC Rev: GGGGAAGAGGATGAAGGATGAGC	

(ThermoFisher Scientific) and specific primer pairs (Table 2) using QuantStudio 12 k Flex instrument (Applied Biosystems). Target gene expression data (Ct) were normalized to RPL10a gene Ct values (ΔCt) and fold change was calculated as $2^{-\Delta\Delta Ct}$.

3.6. Sanger sequencing

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega) and amplified by PCR using specific primer pairs (Table 2). Amplicons were sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3500 Genetic Analyzer (Applied Biosystems).

3.7. STR analysis

The genetic STR profile was obtained using ChromoQuant SuperSTaR Optima QF-PCR Kit (CyberGene AB) detecting 22 STR loci according to the manufacturer's instructions. Amplicons were run on ABI Prism 3500 Genetic Analyzer and fragment lengths determined by Gene Mapper v.4 software (Applied Biosystems).

3.8. Mycoplasma detection

Mycoplasma test was performed using the *N*-GARDE Mycoplasma Detection PCR Kit (Euroclone) following the manufacturer's instructions. Amplicons were run on 1.5 % agarose gel together with positive and negative control samples.

Resource table.

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.2022.103008.

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