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Lab resource: Stem Cell Line

Generation and characterization of a human iPSC line from an ALS patient carrying the Q66K-MATR3 mutation

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A B S T R A C T

Fibroblasts isolated from an Amyotrophic Lateral Sclerosis (ALS)-patient carrying a mutation in Matrin-3 (p.Q66K -MATR3) gene were reprogrammed to the pluripotency stage by using non-integrating episomal plasmids. We generated the Q66K#44DRM induced pluripotent stem cell (iPSC) line that showed regular karyotype, expressed pluripotency-associated markers and were able to properly differentiate into the three germ layers. The heterozygous missense mutation in the MATR3 gene (p.Q66K), which is associated to ALS disease, was present in the generated iPSC line.

Resource table

Unique stem cell line identifier	CIBIOi001-A
Alternative name(s) of stem cell line	*Q66K#44DRM*
Institution	CIBIO, University of Trento, Italy
Contact information of distributor	Alessandro Provenzani *alessandro.provenzani@unitn.it*
Type of cell line	*iPSCs* (induced pluripotent stem cells)
Origin	Human
Additional origin info	Age:51 Sex: Male Ethnicity if known: European, Italian.
Cell source	Patient derived fibroblasts
Clonality	Clonal, clone number 44
Method of reprogramming	Episomal *iPSC* Reprogramming Plasmid
Genetic modification	NO
Type of modification	Spontaneous mutation (missense)
Associated disease	Amyotrophic Lateral Sclerosis (ALS)
Gene/locus	*MATR3/5q31.2*
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	16/01/2018
Cell line repository/bank	N/A
Ethical approval	Patient signed informed consent, number of protocol P/740/CE/2012 released by the ethical committee of the Catholic University School of Medicine

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Resource utility

MATRIN3 (MATR3) mutations have been associated to familial Amyotrophic Lateral Sclerosis (ALS) disease (Johnson et al., 2014; Marangi et al., 2017). We reprogrammed ALS patient-derived fibroblasts carrying p.Q66K-MATR3 mutation to generate a hiPSC line. These patient-derived iPSCs are an useful cellular system for modelling neurodegenerative mechanisms linked to a mutated form of MATR3.

Resource details

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the loss of motor neurons functionality, which leads to muscle atrophy and progressive paralysis, typically leading to death due to respiratory failure within 2–5 years from diagnosis (Hardiman et al., 2017). ALS is categorized in sporadic forms (sALS) (90–95% of cases) which have no obvious genetically inherited component and familial ALS (fALS) (5–10%) associated to genetic dominant inheritance factors (Hardiman et al., 2017). Mutations in *MATR3*-gene were found to be associated to fALS and sALS cases (Johnson et al., 2014; Marangi et al., 2017). MATR3 is an RNA- and DNA-binding protein interacting with TDP-43, a protein linked to ALS and frontotemporal dementia (Johnson et al., 2014). To date some mutations including, Q66K, S85C, F115C, P154S and T622A10, were reported to be ALS-associated (Boehringer et al., 2017) (Table 1).

Fibroblast cells, carrying p.Q66K mutation in MATR3 gene, were isolated from a 51-years old male ALS patient biopsy. iPSCs were generated by a non-integrating system, *oriP/EBNA1*-based episomal plasmid mix (System Biosciences SBI) expressing a combination of reprogramming factors OCT4, SOX2, KLF4, Lin28, p53shRNA, L-MYC and miR-302/367 cluster. Moreover, the expression of a GFP-marker allowed monitoring of plasmid delivery and loss. PCR analysis, performed at the 10th cellular passage, using validated primers (Table 2), showed that constructs used for reprogramming of original fibroblasts and EBV-related latency elements (*EBNA1*) were eliminated from the established iPSC (Fig. 1E). iPSC-like colonies with clear margins and compact cells with high nucleus/cytoplasmic ratio appeared in cultures 15 days following transfection and were picked 10 days later (Fig. 1B). Several clones were isolated and expanded in culture. Among them, iPS Q66K#44DRM cells were expanded over several passages and processed for further characterization, and compared to the commercially available iPS cell line A18945 as positive control. Immunofluorescence (Fig. 1B) and qPCR (Fig. 1C) analyses were performed after the loss of episomal vectors to confirm the expression of endogenous pluripotency markers, including NANOG, OCT4, SOX2 and KFL4. Q66K#44DRM line displayed a normal diploid 46, XY karyotype, without noticeable abnormalities (Fig. 1A) and the presence of heterozygous mutation harboured by parental fibroblasts was confirmed by sequencing in the generated iPSC line in comparison with iPSCs obtained from an healthy volunteer (HV) (Fig. 1D). The genetic matching of the generated iPSC line to the parental fibroblasts was confirmed by short tandem repeat (STR) analysis, showing that genomic DNA extracted from Q66K#44DRM and the parental fibroblasts perfectly matched on the 19 loci tested. Pluripotency competence of Q66K#44DRM was assessed by embryoid bodies formation assay (Fig. 1G). Presence of the three germ layer derivatives in the generated Embryoid bodies was shown by specific markers expression by qPCR (ectoderm markers: NESTIN and βIII-Tubulin; mesoderm marker: α-SMA; endoderm marker: AFP; (Fig. 1F)) and by immunofluorescence staining for βIII-Tubulin, αSMA and GATA-4 (Fig. 1G).

Materials and methods

Cell culture

Patient-derived and healthy volunteer (HV)-derived fibroblasts were provided by the ALS Center of the NEMO Clinical Center (Rome, Italy). Fibroblasts were cultured in DMEM (Sigma-Aldrich) medium containing 20% of Fetal Bovine Serum (FBS), penicillin/streptomycin and Glutamax (Sigma-Aldrich). The human episomal iPSC line A18945 is a commercially available strain derived from CD34+ cord blood using a three-plasmid, seven-factor (SOKMNLT; SOX2, OCT4 (POU5F1), KLF4, MYC, NANOG, LIN28, and SV40L T antigen) EBNA-based episomal system (Thermo Fisher catalog number A18945). Reprogrammed iPSCs and A18495 iPSCs were grown in TeSR-E8 (STEMCELL) medium on Geltrex (Thermo Fisher Scientific)-coated plates (Costar).

iPSC reprogramming

Patient's and HV's fibroblasts were reprogrammed using the Episomal iPSC Reprogramming Plasmids (SBI System Biosciences), following manufacturer's indications. Cells were electroporated with the Amaxa Nucleofector 2D (Lonza), using the P-022 program and seeded on Geltrex-coated wells in complete DMEM medium. The following day, fibroblast medium was replaced with N2B27 medium (DMEM/F12 with Hepes, N2 1%, B27 2%, MEM Non-Essential Aminoacids, Glutamax, β-mercaptoethanol 55 μM), supplemented with FGF-2 (100 ng/ml, STEMCELL). Medium was refreshed every other day

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel B
Phenotype	Qualitative analysis (immunofluorescence)	OCT4, KLF4, SOX2	Fig. 1 panel B
	Quantitative analysis (RT-qPCR)	SOX2, OCT4, NANOG	Fig. 1 panel C
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450	Fig. 1 panel A
Identity	Microsatellite PCR (mPCR)OR	Not performed	
	STR analysis	19	Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	Heterozygous mutation in MATRIN3 gene	Fig. 1 panel D
Microbiology and virology	Mycoplasma	Mycoplasma testing by colorimetric microplate-based assay	Supplementary file
Differentiation potential	Embryoid body formation.	EB morphology: Phase contrast Ectoderm: β III Tubulin Mesoderm: αSMA	Fig. 1 panel G
	Qualitative analysis (immunofluorescence)	Endoderm: GATA-4	
	Embryoid body formation.	Ectoderm: βIII-Tubulin - Nestin Mesoderm: α SMA	Fig. 1 panel F
	Quantitative analysis (RT-qPCR)	Endoderm: AFP	
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not done	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	Not done	N/A
	HLA tissue typing	Not done	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse anti-Sox2 (E-4)	1:100	Santa Cruz sc-365823
	Rabbit anti-KLF4	1:200	Genetex GTX101508
	Mouse anti-OCT4 (C-10)	1:100	Santa Cruz sc-5279
Differentiation markers	Mouse anti-βIII-Tubulin	1:100	Santa Cruz sc-80005
	αSMA	1:1000	Sigma A2547-100UL
	GATA-4	1:500	ThermoFisher PA1-102
Secondary antibodies	Donkey anti-Mouse IgG (H + L), Alexa Fluor 546	1:500	ThermoFisherA10036
	Donkey anti-Rabbit IgG (H + L), Alexa Fluor 488	1:500	ThermoFisher A-21206

Primers		
	Target	Forward/Reverse primer (5′-3′)
Episomal plasmids (PCR)	Episomal Plasmid Detection Primers	Fw: 5′-AGGTCCCTCGAAGAGGTTCA-3′ Rev: 5′-TTCCAACGCGAG AAGGTGTT-3′
Pluripotency markers (qPCR)	Sox2	Fw: 5′-GCTACAGCATGATGCAGGACCA −3′ Rev: 5′- TCTGCGAGCTGGTCATGGAGTT-3′
	Oct4	Fw: 5′-GGAAGGAATTGGGAACACAAAGG-3′ Rev: 5′- AACTTCACCTTCCCTCCAACCA-3′
	Nanog	Fw: 5′-CCTGTGATTTGTGGGCCTG-3′ Rev: 5′- GACAGTCTCCGTGTGAGGCAT-3′
Differentiation potential (qPCR)	Nestin	Fw: 5′-GGAGAAGGACCAAGAACTG-3′ Rev: 5′-ACCTCCTCTGTGGCATTC-3′
	β III Tubulin	Fw: 5′-TCAGCGTCTACTACAACGAGGC-3′ Rev: 5′-GCCTGAAGAGATGTCCAAAGGC-3′
	AFP	Fw: 5′-GCAGAGGAGATGTGCTGGATTG-3′ Rev: 5′-CGTGGTCAGTTTGCAGCATTCTG-3′
	αSMA	Fw: 5′- AATGCAGAAGGAGATCACGG-3′ Rev: 5′- TCCTGTTTGCTGATCCACATC-3′
House-keeping genes (qPCR)	RPLP0	Fw: 5′- CATTCTCGCTTCCTGGAG-3′ Rev: 5′- CTTGACCTTTTCAGCAAGTGG-3′
Genotyping	N/A	
Targeted mutation analysis/sequencing	Matrin3	Fw: 5′- ATGTCCAAGTCATTCC-3′ Rev: 5′- GAACTGGCAAATCACATATAG-3′

for 2 weeks. When colonies with iPSC-like phenotype started to appear, N2B27 medium was replaced with TeSR-E8. After 28 days from the nucleofection, colonies with a clear hiPSCs morphology were picked and expanded. Q66K#44DRM clone and HV clone had been selected for further characterization.

In vitro differentiation

Embryoid bodies (EBs) were obtained and grown in TeSR-E8 (Voden) and Essential 6 (Thermo Fisher Scientific) as previously reported (Cardano et al., 2016).

RNA isolation and PCR and qPCR analyses

RNA was isolated using Trizol Reagent (Thermo Fisher Scientific) and the cDNA was synthesized from 0.5 μg of total RNA using the RevertAid-RT Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Real time PCR was performed using SensiFAST SYBR (Bioline) on a CFX96 Real Time Thermalcycler (BioRad). PCR amplification was carried out to confirm the loss of episomal vector in stabilized Q66K#44DRM line using Phusion High-Fidelity DNA Polymerase (NEB) and specific primers (Table 2). Primers for *OCT4, SOX2* and *NANOG* do not distinguish between endogenous and episomal derived genes. *RPLP0* was used as normalization gene.

Immunofluorescence assay

Cells were fixed with PFA 4%, permeabilized with 0,1% Triton X-100 solution and blocked for 1 h at room temperature in blocking solution (0,1% Triton X-100, 10% FBS in PBS). Samples were incubated overnight at 4 °C with primary antibodies (Table 2) and then signal was revealed with appropriate AlexaFluor secondary antibodies. Hoechst 33258 1 μg/ml (Thermo Fischer Scientific) was used to label the nuclei. Images were acquired with the microscope Leica DM IL Led Fluo and Leica DFC450 C camera (Leica Microsystem).

Sequencing

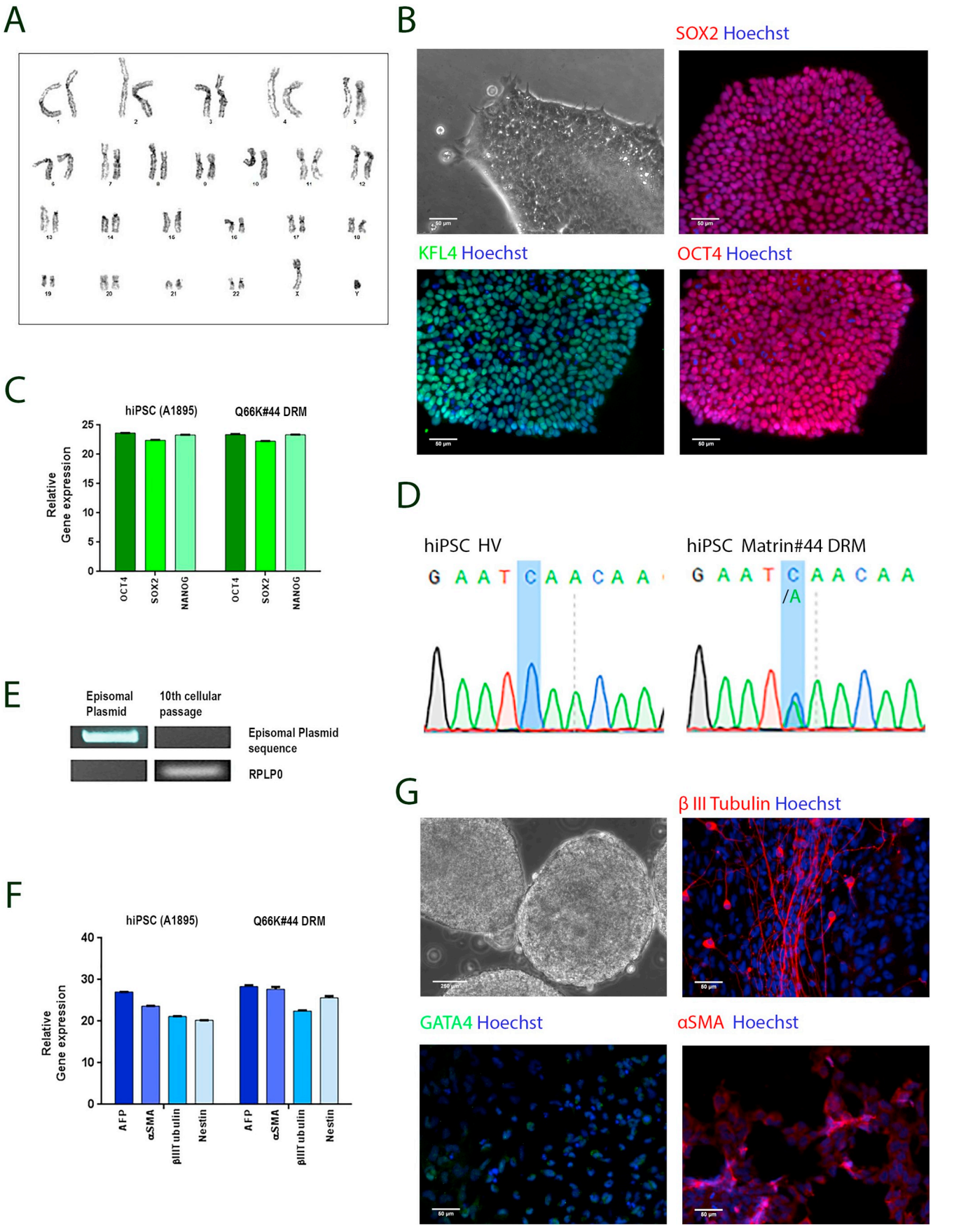
The presence of p.Q66K mutation in *MATR3* gene in the Q66K#44DRM hiPSC, parental fibroblast and HV hiPSC genomes was confirmed by sequencing (Eurofins Genomics) of exon 1. Total RNA was extracted from both cell types and retro-transcribed as described above. The sequence was amplified by PCR using specific primers (Table 2). Amplicons were electrophoretically separated into 1% agarose gel and purified with QIAquik Gel Extraction Kit (Qiagen).

STR analyses

Microsatellites amplification was carried out by amplifying DNA with two multiplexes (one for chromosomes 13, 18 and 21 and the other for the X and Y chromosomes; Devyser Compact v3). PCR products were separated in ABI3100 capillary genetic analyser and results were analysed by ABI Genotyper software.

Karyotyping

hiPSCs were incubated overnight with Colcemid (0.05 mg/ml, Invitrogen) and then dispersed with 0.5% trypsin- 0.02% EDTA (Invitrogen). Cells were subsequentially washed with PBS and treated with 0.075 M KCl hypotonic solution and FBS for 20 min at 37 °C. The



(caption on next page)

Fig. 1. (A) Karyotype analysis of Q66K#44 after reprogramming showed normal diploidy. (B) Immunofluorescent staining showing the expression of the stemness marker proteins, SOX2 (red), KLF4 (green), OCT4 (red), in Q66K#44 DRM cells. (C) qPCR evaluating the expression level of the stemness markers genes *OCT4, SOX2* and *NANOG* in Q66K#44 DRM and in a commercial hiPSC line named A18945. (D) Q66K mutation was confirmed by sequencing after the episomal reprogramming of patient derived fibroblast in Q66K#44 DRM cells, and was compared with iPSCs obtained from a Healthy Volunteer (hiPSC HV). (E) PCR showing the loss of expression of the episomal vector in Q66K#44 DRM cells at the 10^{th} passage. (F) qPCR evaluating the expression level of marker genes belonging to the three germ layers in embryoid bodies obtained from Q66K#44 DRM hiPSCs. *AFP* (Endoderm), *αSMA* (Mesoderm), *βIIITubulin* (Ectoderm) and *Nestin* (Ectoderm). (G) Immunofluorescent staining showing the protein expression of marker genes belonging to the three germ layers in embryoid bodies obtained from Q66K#44 DRM hiPSCs. AFP (Endoderm), αSMA (Mesoderm), βIIITubulin (Ectoderm) and Nestin (Ectoderm).

cells were then fixed with methanol:acetic acid (3:1, *v*/v) solution and stored for 1 h at −20 °C. Cell suspension were dropped on ice glass slides and stained in Giemsa stain after banding with GAG-acid solution at 55 °C. Karyotyping was performed at the resolution of 450 banding by use of the Genikon software (Nikon Italia) and described in accordance to the ISCN 2016: An International System for Human Cytogenomic Nomenclature (2016) ISBN: 978-3-318-05857-4.

Mycoplasma test

Mycoplasma contamination was checked by colorimetric mycoplasma detection assay (Invivogen) following manufacturer's indications.

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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.2018.10.011.

References

Boehringer, A., Garcia-Mansfield, K., Singh, G., Bakkar, N., Pirrotte, P., Bowser, R., 2017. ALS associated mutations in matrin 3 alter protein-protein interactions and impede mRNA nuclear export. Sci. Rep. 7 (1), 1–14. https://doi.org/10.1038/s41598-017-14924-6.

Cardano, M., Marsoner, F., Marcatili, M., Karnavas, T., Zasso, J., Lanterna, L.A., Conti, L., 2016. Establishment of induced pluripotent stem cell (iPSC) line from 55-year old male patient with hemorrhagic Moyamoya disease. Stem Cell Res. 17 (3), 623–626. https://doi.org/10.1016/j.scr.2016.11.006.

Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E.M., Logroscino, G., Robberecht, W., ... van den Berg, L.H., 2017. Amyotrophic lateral sclerosis. Nat. Rev. Dis. Prim. 3, 17085. https://doi.org/10.1038/nrdp.2017.85.

Johnson, J.O., Pioro, E.P., Boehringer, A., Chia, R., Feit, H., Renton, A.E., ... Traynor, B.J., 2014. Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. Nat. Neurosci. 17 (5), 664–666. https://doi.org/10.1038/nn.3688.

Marangi, G., Lattante, S., Doronzio, P.N., Conte, A., Tasca, G., Monforte, M., ... Sabatelli, M., 2017. Matrin 3 variants are frequent in Italian ALS patients. Neurobiol. Aging 49 (218). https://doi.org/10.1016/j.neurobiolaging.2016.09.023.