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To cite this version:

Susie Barbeau, Romain Desprat, Bruno Eymard, Cécile Martinat, Jean-Marc Lemaitre, et al.. Generation of a human induced pluripotent stem cell line (iPSC) from peripheral blood mononuclear cells of a patient with a myasthenic syndrome due to mutation in COLQ. Stem Cell Research, Elsevier, 2020, 49, pp.102106. 10.1016/j.scr.2020.102106 . hal-03146055

HAL Id: hal-03146055

https://hal.sorbonne-universite.fr/hal-03146055

Submitted on 18 Feb 2021
Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell line (iPSC) from peripheral blood mononuclear cells of a patient with a myasthenic syndrome due to mutation in COLQ

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A B S T R A C T

Congenital myasthenic syndromes (CMS) are a class of inherited disorders affecting the neuromuscular junction, a synapse whose activity is essential for movement. CMS with acetylcholinesterase (AChE) deficiency are caused by mutations in COLQ, a gene that codes for a synaptic non-fibrillar collagen (Legay, 2018). ColQ is synthetized by muscle cells and anchors AChE in the synaptic cleft where AChE hydrolyses acetylcholine to control neurotransmission. To investigate the molecular and cellular defects created by a ColQ C-terminus mutation in human muscle cells and in neuromuscular junction in vitro, we have generated hiPS cells from a patient’s Peripheral Blood Mononuclear cells (PBMCs) by reprogramming these cells using a non-integrative method using Sendai viruses bearing the four Yamanaka factors Oct3/4, Sox2, KLF4, and L-Myc.

1. Resource table

| Unique stem cell line identifier | REGUi009-A |
|----------------------------------|------------|
| Institution                      | Saints-Peres Paris Institute for the Neurosciences (SPPIN) |
| Contact information of distributor | Pr. Claire Legay, claire.legay@parisdescartes.fr |
| Type of cell line                | human induced Pluripotent Stem Cell (hiPSC) |
| Origin                           | Human |
| Additional origin info           | Male, 28 years old |
| Cell Source                      | Peripheral Blood Mononuclear cells (PBMCs) |
| Clonality                        | Mixed |
| Method of reprogramming          | Sendai virus expressing OCT4, SOX2, L-MYC, KLF4 genes |
| Genetic Modification              | Congenital |
| Associated disease               | Congenital Myasthenic Syndrome with AChE deficiency |
| Gene/locus                       | COLQ/1281C>T |
| Date archived/stock date         | February 2020 |
| Cell line repository/bank        | No |
| Ethical approval                 | N’AC-2018-3156 |

2. Resource utility

Congenital Myasthenic Syndrome (CMS) with AChE deficiency correspond to a rare disease due to mutations in COLQ, a gene that codes for a synaptic non-fibrillar collagen (Legay, 2018). ColQ is synthetized by muscle cells and anchors AChE in the synaptic cleft where AChE hydrolyses acetylcholine to control neurotransmission. To investigate the molecular and cellular defects created by a ColQ C-terminus mutation in human muscle cells and in neuromuscular junction in vitro, we have generated hiPS cells from patient with a COLQ 1281C>T homozygous mutation. To our knowledge, this is the first hiPSC line produced from CMS with AChE deficiency patient (see Table 1).

3. Resource details

In this study we have generated hiPS cells named REGUi009-A from a 28 years old male patient with a COLQ 1281C>T homozygous mutation (Fig. 1 A and C). The patient mutation and pathology have been described in (Wargon et al., 2012), and the patient referred as patient 2 in this publication. The blood sample for the Peripheral Blood Mononuclear Cells (PBMCs) purification was collected in heparin tubes. Another blood sample was collected to extract the patient genomic DNA. The PBMCs reprogramming was done using non-integrative CytoTune™-iPS 2.1 Sendai Reprogramming kit composed by the four Yamanaka factors Oct3/4, Sox2, KLF4, and L-Myc at the SAFE-iPSC Core facility.

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https://doi.org/10.1016/j.scr.2020.102106
Received 27 October 2020; Received in revised form 17 November 2020; Accepted 27 November 2020
Available online 2 December 2020
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The PBMCs were isolated from the whole blood sample by the Genethon core facility using Ficoll density gradient cell separation.

4.2. Ethics statement

The patient signed a written informed consent and the study was approved by the scientific ethical committee “Comité de protection des personnes Ile-de-France XI”, under the authorisation n° AC-2018–3156.

4.3. hiPSC generation

Prior to reprogramming, PBMCs were cultured 5 days in erythroid media: SFEM II medium completed with erythroid expansion supplement (Stemcell Technologies #09655 and #02692). The PBMCs reprogramming was done according to CytoTune®-iPS 2.1 (Invitrogen #A34546). Briefly, 5 × 10⁴ cells in SFEM II were added together with the sendai viruses KOS, L-Myc and Kif4 respectively at 10, 10 and 6 MOI. The tube containing cells and viruses was centrifugated for 1 h at 2250 rpm. Spinoculated cells were plated in erythroid media, with 5 mM Rock inhibitor and 100 mM sodium butyrate under hypoxic conditions for 7 days. From day 3, the cells were cultivated on matrigel coated dishes.

4.4. Alkaline phosphatase activity

The cells were stained with Vector® Red AP substrate (Vector Laboratories kit #SK-5100) according to manufacturer’s instructions. The culture media was discarded before incubation with the substrate working solution for 20–30 min in the dark.

4.5. Immunofluorescence labelling

Cells grown on coverslips were fixed in 4% PBS/paraformaldehyde and labelled overnight at room temperature, after a 60-minute incubation in the blocking buffer (5% goat serum) supplemented with 0.1% Saponin according to the standard protocol of StemLight® Pluripotency Antibody Kit (Cell Signaling). Cells were incubated with the appropriate fluorochrome-conjugated secondary antibodies for 60 min. DNA was stained with DAPI (ImmunoChemistry, #6244) for 15 min. Image acquisition was performed with an Axio Imager Z1(ZEISS) Apotome, X10 objective (Fig. 1.E) and a confocal microscope Zeiss LSM880, X40 objective (Fig. 1.F).

4.6. Fluorescence-activated cell sorting (FACS)

BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit was used for FACS. Cells were analysed on a CANTO II Becton Dickinson and analysis was made with Flow-JO.

4.7. hiPSC karyotyping

30 metaphases were counted and 6 cells were karyotyped for the M-FISH analyses. SNP analysis was carried out following the Illumina Infinium Core24 protocol (Integragen) and results analysed on Genome Studio software v2011.1.
4.8. Three germ layer in vitro differentiation and characterization

Embryoid bodies (EBs) were formed in suspension, in low attachment 96 wells plates in E8 medium completed with polyvinyl alcohol (PVA) 4 mg/ml and 10 µM Rock inhibitors Y27632 based on published protocol (Lin and Chen, 2014). The next day, medium was changed to E6 medium and EBs were left to differentiate spontaneously. After 7 days, EBs were plated on a matrigel coated µ-dishes (ibidi #81156) to adhere and cells started to migrate. To promote endoderm differentiation, some EBs were treated with Activin-A 100 ng/ml (R&D systems #338-AC) during 3 days when in suspension, and the first 4 days in adhering culture. Cells were fixed after 10 days of adhering culture and

Fig. 1. Characterization of REGU009-A hiPSC line bearing COLQ c.1281 C>T mutation responsible for a CMS.
Table 2
Reagents details.

| Antibodies used for immunocytochemistry/flow-cytometry | Antibody | Dilution | Company Cat # and RRID |
|-------------------------------------------------------|----------|----------|------------------------|
| Pluripotency markers immunostaining                    | Oct-4A Rabbit mAb (Clone C30A3) IgG | 1/200 | Cell signaling technology #2840, RRID: AB_2167691 |
| Pluripotency markers immunostaining                    | Sox2 XP® Rabbit mAb (Clone D6D9) IgG | 1/200 | Cell signaling technology # 3579, RRID: AB_2195767 |
| Pluripotency markers immunostaining                    | Nanog XP® Rabbit mAb (Clone D73G4) IgG | 1/200 | Cell signaling technology # 4903, RRID: AB_10559205 |
| Pluripotency markers immunostaining                    | SSEA4 Mouse mAb (Clone M8213) IgG | 1/200 | Cell signaling technology # 4755, RRID: AB_1264259 |
| Pluripotency markers immunostaining                    | TRA-1–60(5) IgM Mouse mAb (Clone TRA-1–60(5)) IgM | 1/200 | Cell Signaling Technology # 4746, RRID: AB_2119059 |
| Pluripotency markers immunostaining                    | TRA-1–81 Mouse mAb (Clone TRA-1–81) IgM | 1/200 | Cell signaling technology # 4745, RRID: AB_2119060 |
| Secondary Antibody                                     | Alexa Fluor® 488 conjugate Goat anti-Rabbit IgG | 1/400 | Fisher scientific # A-11034, RRID: AB_2576217 |
| Secondary Antibody                                     | Alexa Fluor® 555 conjugate Goat anti-Rabbit IgG | 1/400 | Invitrogen-thermofisher scientific # A21424, RRID: AB_141780 |
| Pluripotency markers flow cytometry                     | PE Mouse anti-human Nanog (Clone: N31-355) | 1/5 | BD Biosciences Cat#560791, RRID: AB_1937305 |
| Pluripotency markers flow cytometry                     | PerCP-CyTM 5.5 Mouse anti-Oct3/4 (Clone: 40/ Oct-3) | 1/5 | BD Biosciences Cat#560794, RRID: AB_1937313 |
| Pluripotency markers flow cytometry                     | Alexa Fluor R 647 Mouse anti-Sox2 (Clone:245,610) | 1/5 | BD Biosciences Cat#560301, RRID: AB_1645308 |
| Pluripotency markers flow cytometry                     | Alexa Fluor R 647 Mouse anti-SSEA-4 (Clone: MCS13–70) | 1/5 | BD Biosciences Cat#560796, RRID: AB_2033991 |
| Pluripotency markers flow cytometry                     | PE Mouse IgG1, k Isotype Control (Clone: MOPC-21) | 1/5 | BD Biosciences Cat#554121, RRID: AB_395252 |
| Pluripotency markers flow cytometry                     | PerCP-Cy5.5 Mouse IgG1, x Isotype Control (Clone: X40) | 1/5 | BD Biosciences Cat#347202, RRID: AB_400265 |
| Pluripotency markers flow cytometry                     | Alexa Fluor® 647 Mouse IgG2a, x Isotype Control (Clone: MOPC-173) | 1/5 | BD Biosciences Cat#558020, RRID: AB_396989 |
| Differentiation markers immunostaining                 | Chicken anti-TUJ1 | 1:1500 | Abcam #ab41489, RRID: AB_727049 |
| Differentiation markers immunostaining                 | Mouse anti-AFP | 1:200 | Abcam #ab9380, RRID: AB_304203 |
| Differentiation markers immunostaining                 | Rabbit anti-SMA | 1:100 | Abcam #ab5694, RRID: AB_2223021 |
| Secondary Antibody                                     | Alexa Fluor® 488 conjugate Goat anti-Chicken IgG | 1:500 | Invitrogen-thermofisher scientific # A10520, RRID: AB_2534029 |
| Secondary Antibody                                     | Goat anti-Rabbit IgG (H + L, Cyanine3) | 1:500 | Invitrogen-thermofisher scientific # A21052, RRID: AB_2535719 |
| Secondary Antibody                                     | Goat anti-Mouse IgG (H + L, Alexa Fluor 633) | 1:500 | Invitrogen-thermofisher scientific # A21052, RRID: AB_2535719 |

4.9. Authentication of hiPSC identity

hiPSC identity was confirmed by short tandem repeat (STR) analysis by Eurofins Genomics.

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.

Acknowledgements

The authors would like to thank Dr Damien Sternberg for sharing genetic information on the patient, Saffaa Saker and Angéligue Dupont (Génethon) for purification of the PBMC, and Karine Giraud-Tribout and Lina El-Kassar for technical help (I-STEM core facility). This work was supported by the Agence Nationale de la Recherche (ANR-15-CE14-0022-01 HUMANISM).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2020.102106.

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