



**HAL**  
open science

# 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

Susie Barbeau, Romain Desprat, Bruno Eymard, Cécile Martinat, Jean-Marc Lemaitre, Claire Legay

## ► 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*, 2020, 49, pp.102106. 10.1016/j.scr.2020.102106 . hal-03146055

**HAL Id: hal-03146055**

**<https://hal.sorbonne-universite.fr/hal-03146055v1>**

Submitted on 18 Feb 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

Susie Barbeau<sup>a</sup>, Romain Desprat<sup>b</sup>, Bruno Eymard<sup>c</sup>, Cécile Martinat<sup>d</sup>, Jean-Marc Lemaître<sup>b,e</sup>, Claire Legay<sup>a,\*</sup>

<sup>a</sup> Paris University, SPPIN CNRS 8003 Laboratory, Paris, France

<sup>b</sup> SAFE-iPSC Facility INGESTEM, Univ. Montpellier, CHU de Montpellier, France

<sup>c</sup> Paris-Est Neuromuscular Center, Institut de Myologie, Hôpital Pitié-Salpêtrière, Paris, France

<sup>d</sup> INSERM UEVE UMR 861, Paris Saclay Univ. I-STEM, 91100 Corbeil-Essonnes, France

<sup>e</sup> Institute for Regenerative Medicine and Biotherapy, INSERM UMR1183, Univ Montpellier, Montpellier, France

### ABSTRACT

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 collagen that anchors AChE in the synapse. To study the pathophysiological mechanisms of the disease in human cells, we have generated iPSC from a patient's Peripheral Blood Mononuclear cells (PBMC) 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, <a href="mailto:claire.legay@parisdescartes.fr">claire.legay@parisdescartes.fr</a>
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 synthesized 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<sup>TM</sup>-iPS 2.1 Sendai Reprogramming kit composed by the four Yamanaka factors Oct3/4, Sox2, Klf4, and L-Myc at the SAFE-iPS Core facility

\* Corresponding author.

E-mail address: [claire.legay@parisdescartes.fr](mailto:claire.legay@parisdescartes.fr) (C. Legay).

<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

1873-5061/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

**Table 1**  
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	REGUi009-A appear normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for pluripotency: Oct4, Nanog, SOX2, SSEA4 Phosphatase alkaline	Fig. 1 panel E Fig. 1 panel A
	Quantitative analysis (Flow cytometry)	Positive for cell surface markers Oct4, Nanog, SOX2, and SEEA-4Tra1-60, Tra1-81	Fig. 1 panel D
Genotype	Karyotype (M-FISH) and resolution	46XY, 400	Fig. 1 panel B
Identity	SNP analysis	5 sites tested, genotype conformity is normal	Suppl. Fig. S1a and S1b
	Stemgenomics iCS-digital™ Pluri test STR analysis	no abnormalities detected 16 independent loci tested: hiPSC genetically identical to PBMC	
Mutation analysis (if applicable)	Donor mutation description (From electropherogram data)	COLQ gene carrying c.1281C>T mutation	Fig. 1 panel C
	Sequencing Southern Blot OR WGS	N/A N/A	
Microbiology and virology	Mycoplasma testing by luminescence (MycoAlert Kit, Lonza)	REGUi009-A is negative	Suppl. Fig. S2
Differentiation potential	Embryoid body formation	Ectoderm, endoderm, and mesoderm expression detected by immunocytochemistry	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

of the Institute for Regenerative Medicine & Biotherapy (IRMB). hiPS cells REGUi009-A were cultured on matrigel coated plate in E8 medium (Fig. 1 A, right panel), passaged with Versene solution (Gibco #15040066), and frozen in Cryostor CS10 preservation media (Sigma Aldrich #C2874). Karyotyping of REGUi009-A cells by M-FISH (Fig. 1B) show all cells had 46 chromosomes and XY sex chromosomes as expected for a male patient. SNP analysis detected a duplication in chromosome 1 p36.21 zone in both REGUi009-A cells and somatic cells used for reprogramming (Fig S1a and S1b). Karyotyping and genotyping of REGUi009-A were performed by M-FISH and SNP analysis at the I-STEM core facility. Moreover, the iCS-digital™ PSC test (Stemgenomics, (Assou et al., 2020)) detected no genomic abnormalities on REGUi009-A cells. The phosphatase alkaline activity was confirmed by the presence of a precipitate with Vector Red AP substrate kit (#SK-5100, Fig. 1A). The expression by REGUi009-A of Tra1-60, Tra1-81, and SEEA-4 cell surface markers and Oct4, Nanog, and Sox2 pluripotency-associated transcription factors was assessed by FACS (Fig. 1D, Table 2) and immunocytochemistry (Fig. 1E, Table 2). In addition, we demonstrated the REGUi009-A capacity to generate the three germ layers. Embryoid bodies (EBs) form in suspension (Fig. 1F, right panel), adhere and migrate on a dish, and express molecules representative of the germ layers, ie ectoderm, endoderm, and mesoderm using respectively  $\beta$ III-tubulin (Tuj1),  $\alpha$ -fetoprotein (AFP) and smooth-muscle actin (SMA) antibodies (Fig. 1F). STR profiles analyses on 16 independent loci found

hiPS cells genetically identical to the patient PBMC (data available from the authors). Absence of mycoplasma contamination (Fig S2) was assessed by luminescence (MycoAlert Mycoplasma Detection Kit, Lonza).

## 4. Materials and methods

### 4.1. Human blood samples

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,  $5 \times 10^4$  cells in SFEM II were added together with the sendai viruses KOS, L-Myc and Klf4 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 (Integrage) 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  $\mu$ M 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  $\mu$ -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

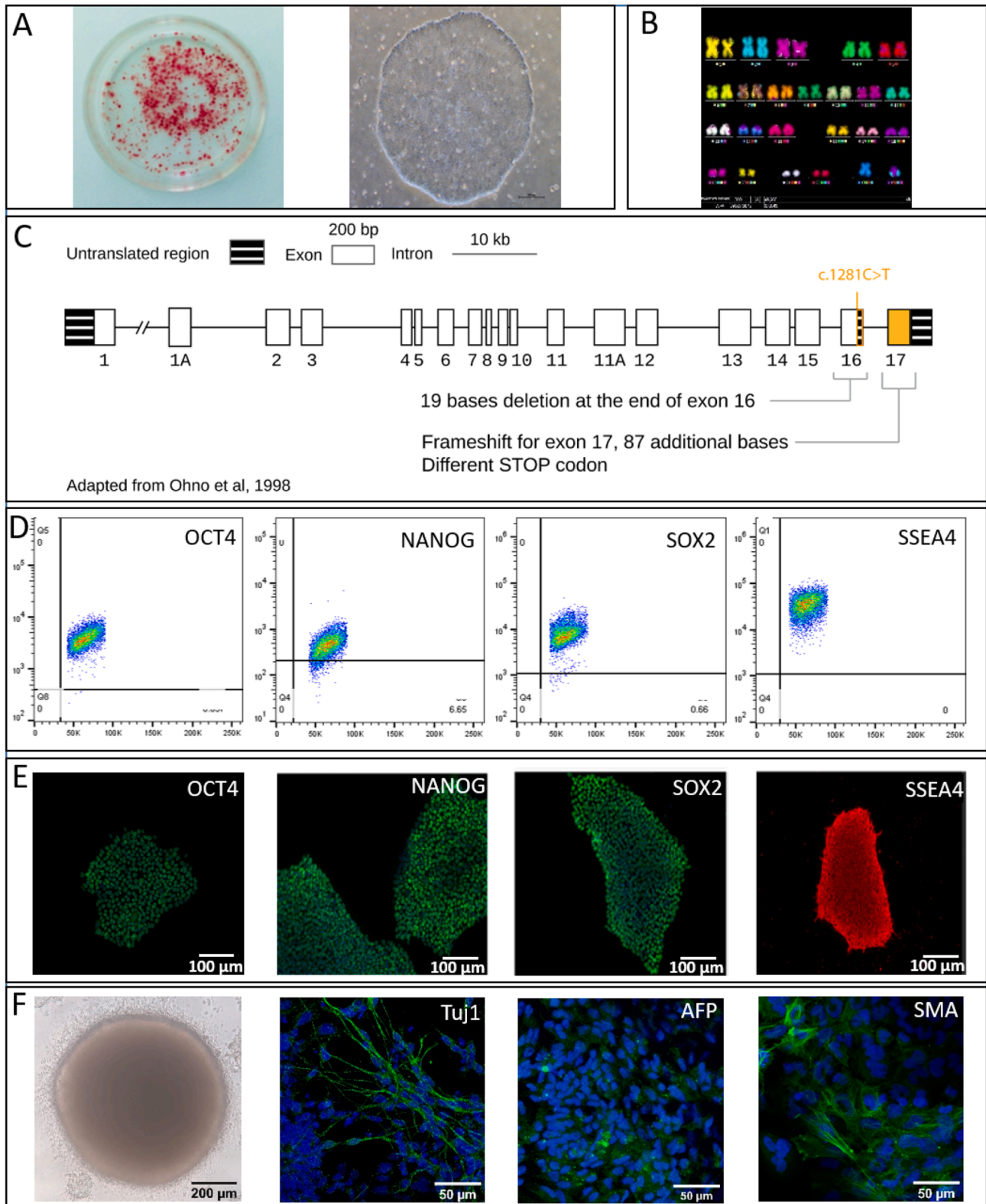


Fig. 1. Characterization of REGUi009-A hiPSC line bearing COLQ c.1281 C>T mutation responsible for a CMS.

**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 MC813) IgG	1/200	Cell signaling technology # 4755, RRID: AB_1264259
Pluripotency markers immunostaining	TRA-1-60(S) IgM Mouse mAb (Clone TRA-1-60(S)) 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 #A-21424, 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 FluorR 647 Mouse anti-Sox2 (Clone:245,610)	1:5	BD Biosciences Cat#560301, RRID: AB_1645308
Pluripotency markers flow cytometry	Alexa FluorR 647 Mouse anti-SSEA-4 (Clone: MC813-70)	1:5	BD Biosciences Cat#560796, RRID: AB_2033991
Pluripotency markers flow cytometry	PE Mouse IgG1, κ Isotype Control (Clone MOPC-21)	1:5	BD Biosciences Cat#554121, RRID: AB_395252
Pluripotency markers flow cytometry	PerCP-Cy5.5 Mouse IgG1, κ Isotype Control (Clone: X40)	1:5	BD Biosciences Cat#347202, RRID: AB_400265
Pluripotency markers flow cytometry	Alexa Fluor® 647 Mouse IgG2a, κ 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 #ab3980, 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	Abcam #ab150169, RRID: AB_2636803
Secondary Antibody	Goat anti-Rabbit IgG (H + L), Cyanine3	1:500	Invitrogen-thermofisher scientific #A10520, RRID: AB_2534029
Secondary Antibody	Goat anti-Mouse IgG (H + L), Alexa Fluor 633	1:500	Invitrogen-thermofisher scientific #A21052, RRID: AB_2535719

immunostained.

#### 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, Safaa Saker and Angélique Dupont (Généthon) for purification of the PBMC, and Karine Giraud-Triboulet 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>.

#### References

- Assou, S., Girault, N., Plinet, M., Bouckenheimer, J., Sansac, C., Combe, M., Mianné, J., Bourguignon, C., Fieldes, M., Ahmed, E., Commes, T., Boueux, A., Lemaître, J.-M., De Vos, J., 2020. Recurrent genetic abnormalities in human pluripotent stem cells: definition and routine detection in culture supernatant by targeted droplet digital PCR. *Stem Cell Reports* 14 (1), 1–8. <https://doi.org/10.1016/j.stemcr.2019.12.004>.
- Legay, C., 2018. Congenital myasthenic syndromes with acetylcholinesterase deficiency, the pathophysiological mechanisms: CMS with AChE deficiency. *Ann. N.Y. Acad. Sci.* 1413 (1), 104–110. <https://doi.org/10.1111/nyas.13595>.
- Lin, Y., Chen, G., 2014. Embryoid body formation from human pluripotent stem cells in chemically defined E8 media. *StemBook*. Harvard Stem Cell Institute, Cambridge (MA).
- Wargon, I., Richard, P., Kuntzer, T., Sternberg, D., Nafissi, S., Gaudon, K., Lebaill, A., Bauche, S., Hantaï, D., Fournier, E., Eymard, B., Stojkovic, T., 2012. Long-term follow-up of patients with congenital myasthenic syndrome caused by COLQ mutations. *Neuromuscular Disorders* 22 (4), 318–324. <https://doi.org/10.1016/j.nmd.2011.09.002>.