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Lab Resource: Multiple Cell Lines

# Generation of six multiple sclerosis patient-derived induced pluripotent stem cell lines

Miquel-Serra L.[a], Duarri A.[a,b], Muñoz Y.[a], Kuebler B.[a], Aran B.[a,b], Costa C.[c], Martí M.[a], Comabella M.[c], Malhotra S.[c], Montalban X.[c], Veiga A.[a,b,d], Raya A.[a,d,e,*]

[a] Center of Regenerative Medicine in Barcelona (CMRB), Barcelona, Spain
[b] National Stem Cell Bank-Barcelona Node, Biomolecular and Bioinformatics Resources Platform PRB2, ISCIII, CMRB, Barcelona, Spain
[c] Department of Neurology-Neuroimmunology, Centre d'Esclerosi Múltiple de Catalunya (Cemcat), Hospital Universitari Vall d'Hebron, Barcelona, Spain
[d] Center for Networked Biomedical Research on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain
[e] Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

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ABSTRACT

Multiple sclerosis (MS) is considered a chronic autoimmune disease of the central nervous system that leads to gliosis, demyelination, axonal damage and neuronal death. The MS disease aetiology is unknown, though a polymorphism of the TNFRSF1A gene, rs1800693, is known to confer an increased risk for MS. Using retroviral delivery of reprogramming transgenes, we generated six MS patient-specific iPSC lines with two distinct genotypes, CC or TT, of the polymorphism rs1800693. iPSC lines had normal karyotype, expressed pluripotency genes and differentiated into the three germ layers. These lines offer a good tool to study MS pathomechanisms and for drug testing.



## Resource table

| | |
|---|---|
| Unique stem cell lines identifier | ESi048-A<br>ESi049-A<br>ESi050-A<br>ESi051-A<br>ESi052-A<br>ESi053-A |
| Alternative names of stem cell lines | MS_FiPS1_R4F_3 (ESi048-A)<br>MS_FiPS2_R4F_10 (ESi049-A)<br>MS_FiPS3_R4F_1 (ESi050-A)<br>MS_FiPS4_R4F_1 (ESi051-A)<br>MS_FiPS5_R4F_6 (ESi052-A)<br>MS_FiPS6_R4F_2 (ESi053-A) |
| Institution | Center of Regenerative Medicine in Barcelona<br>CEMCAT, Hospital Universitari Vall d'Hebron |
| Contact information of distributor | Anna Veiga, aveiga@cmrb.eu |
| Type of cell lines | iPSC |
| Origin | Human |
| Cell Source | Skin fibroblasts |
| Method of reprogramming | Retrovirus (Oct4, Sox2, c-Myc, Klf4) |
| Multiline rationale | Multiple sclerosis non-isogenic cell lines |
| Gene modification | NO |
| Type of modification | N.A. |
| Associated disease | Multiple sclerosis |
| Gene/locus | Unknown. *TNFRSF1A* polymorphism rs1800693 |
| Method of modification | N.A. |
| Name of transgene or resistance | N.A. |
| Inducible/ constitutive system | N.A. |
| Date archived/ stock date | January, 2015 |
| Cell line repository/bank | Registration ongoing at Spanish National Stem Cell Bank: http://www.eng.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-iPS.shtml |
| Ethical approval | Patient informed consent obtained/Ethics Review Board-competent authority approval obtained by Comité de Ética e Investigación Clínica-CEIC-CMRB (ADD06/2014 17/2012)) and by the Catalan Authority for Stem Cell Research (Approval number: 326 191 2) |

* Corresponding author at: Center of Regenerative Medicine in Barcelona (CMRB), Barcelona, Spain.
*E-mail address:* araya@cmrb.eu (A. Raya).

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**Table 1**
Summary of MS iPSC line generated.

| iPSC line names | Abbreviation in figures | Gender | Age | Ethnicity | Genotype *TNFRSF1A* rs1800693 | Disease |
|---|---|---|---|---|---|---|
| MS_FiPS1_R4F_3 (ESi048-A) | MS1-3 | Female | 33 | Caucasian | TT | Multiple Sclerosis |
| MS_FiPS2_R4F_10 (ESi049-A) | MS2-10 | Male | 44 | Caucasian | TT | Multiple Sclerosis |
| MS_FiPS3_R4F_1 (ESi050-A) | MS3-1 | Male | 41 | Caucasian | CC | Multiple Sclerosis |
| MS_FiPS4_R4F_1 (ESi051-A) | MS4-1 | Female | 49 | Caucasian | CC | Multiple Sclerosis |
| MS_FiPS5_R4F_6 (ESi052-A) | MS5-6 | Female | 42 | Caucasian | TT | Multiple Sclerosis |
| MS_FiPS6_R4F_2 (ESi053-A) | MS6-2 | Female | 49 | Caucasian | CC | Multiple Sclerosis |

## Resource utility

Multiple sclerosis (MS) is considered a chronic autoimmune disease of the central nervous system that leads to gliosis, demyelination, axonal damage and neuronal death (Hagemeier et al., 2012). The MS disease aetiology is unknown but both complex genetic and environmental factors contribute to the disease manifestation (Sospedra & Martin, 2016). Particularly, polymorphism of the tumour necrosis factor (TNF) receptor superfamily member 1A (*TNFRSF1A*) gene, rs1800693, confers an increased risk for suffering MS and may act as a disease modifier (Comabella et al., 2013; Gregory et al., 2012). The presence of the risk allele CC results in skipping of exon 6 of *TNFRSF1A* (Δ6-TNF-R1 isoform), which is absent in TT carriers.

## Resource details

Here, dermal fibroblasts were derived from skin biopsies of six MS patients carrying the CC or the TT genotypes (three patients each) of the *TNFRSF1A* polymorphism rs1800693 (Table 1). Fibroblasts were reprogrammed to iPSCs by an integrative method using retroviral transduction of the four Yamanaka factors encoded by two polycistronic vectors (pMX-OS, encoding mouse Oct4 and Sox2; and pMX-KM, encoding Klf4 and c-Myc). MS iPSC lines carrying the TT (lines MS1-3, MS2-10 and MS5-6) or CC genotype (lines MS3-1, MS4-1 and MS6-2) of the TNFRSF1A polymorphism rs1800693 were confirmed by the absence or presence of Δ6-TNF-R1 isoform (Fig. 1A). The lines had normal diploid 46, XX or XY karyotype, without any detectable abnormalities (Fig. 1B). The integration of the transgenes Klf4/c-Myc (pMX-KM) and Oct4/Sox2 (pMX-OS) was determined by PCR (Supplementary Fig. S1A) and silencing of transgenes was confirmed by qRT-PCR (Supplementary Fig. S1B).

The pluripotency of MS iPSC lines was confirmed by the mRNA expression of endogenous human factors SOX2, OCT4, KLF4 and c-MYC (Fig. 1C), alkaline phosphatase activity (Supplementary Fig. S1C) and immunofluorescence analysis of the pluripotency-associated markers OCT4, SOX2, NANOG, TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 (Fig. 1D). The differentiation potential of the MS lines was confirmed by *in vitro* differentiation of embryoid bodies towards the three main embryo germ layers, as judged by immunofluorescence-based detection of the definitive endoderm markers α-fetoprotein (AFP) and forkhead box A2 (FOXA2), ectodermal markers βIII-tubulin (TUJ1) and glial fibrillary acidic protein (GFAP), and mesodermal markers α-smooth muscle actin (ASMA) and α-sarcomeric actin (ASA) (Fig. 1E). The iPSC identity was confirmed by short tandem repeat analysis and compared with the original patient's fibroblasts (Supplementary Table S1). The full characterization is summarized in Table 2.

## Materials and methods

### *Patient fibroblasts and reprogramming*

The study was approved by the ethical committee of the centres involved (Comité de Ética e Investigación Clínica-CEIC-CMRB (ADD06/2014 17/2012)) and by the Catalan Authority for Stem Cell Research (Approval number: 326 191 2). After written consent, skin biopsies were obtained from 6 MS patients in accordance with the guidelines dictated by the Ethics Committee of the Hospital Universitari Vall d'Hebron.

Primary cultures of dermal fibroblasts cells were obtained culturing the dermis in IMDM complete (Iscove's modified Dulbecco's medium (Gibco)) supplemented with 10% FBS (Hyclone) (GE Healthcare Life Sciences), 2 mM Glutamax (Gibco), 1% Non-essential amino acids (Lonza) and 1% penicillin–streptomycin (Gibco) at 37 °C and 5% $CO_2$. Fibroblasts were used for reprogramming by retroviral delivery. Retroviruses were produced in Phoenix Amphotropic cells following transfection with pMX-OCT4_Flag-VP16-PTV-Sox2_HA-Orange (pMX-OS) and pMX-KLF4-cMYC-GFP (pMX-KM) polycistronic vectors. Retrovirus-containing medium was collected 48 h post-transfection and filtered through 0,45 μm filter. Polybrene (10 μg/ml) was added to the medium and used to transduce $1 \times 10^5$ cells for 24 h, then the medium was replaced for DMEM complete for 3 days. Transduced human fibroblasts were trypsinized and seeded onto irradiated human foreskin fibroblasts in HES medium (Knockout DMEM supplemented with 20% Knockout serum replacement, 2 mM Glutamax (Gibco), 1% Non-essential amino acids (Lonza), 1% penicillin–streptomycin (Gibco), 0,1 mM β-mercaptoethanol and 10 ng/ml bFGF) until iPSC colonies appeared. Colonies were manually picked and passaged for expansion. From passage 5, colonies were adapted to matrigel-coated plates and cultured with mTSeR1 (Stem Cell Technologies).

### *Karyotyping*

To evaluate the genomic integrity of the iPSC lines, karyotypes were performed (Ambar, Barcelona). Briefly, 70% confluent iPSC colonies on matrigel were incubated with KaryoMax colcemid (Invitrogen), trypsinized, treated with hypotonic solution and fixed in Carnoy solution (75% methanol, 25% acetic acid). Karyotyping was performed on G-banded metaphase chromosomes following standard procedures. A minimum of 20 metaphases were examined.

### *Alkaline phosphatase, immunocytochemistry for pluripotency and in vitro differentiation*

All procedures have been described previously (Marti et al., 2013). Briefly, to detect alkaline phosphatase (AP) activity, iPSCs were fixed with 4% paraformaldehyde (PFA) for 1 min, washed with PBS and

**Fig. 1.** Characterization of MS iPSC lines.A. mRNA expression levels of the Δ6-TNF-R1 isoform. B. Karyotypes of representative metaphases showing normal 46 chromosomes. C. mRNA expression levels of endogenous pluripotency markers. Ct values were normalized to the geometric mean of GAPDH (mean ± SD). D. Confocal images showing immunodetection of pluripotency markers. Scale bar: 50 μm. G. In vitro differentiation of embryoid bodies using specific antibodies against the endodermal markers α-fetoprotein (AFP) and forkhead box A2 (FOXA2), ectodermal markers βIII-tubulin (TUJ1) and Glial fibrillary acidic protein (GFAP) and mesodermal marker α-smooth muscle actin (ASMA) and α-sarcomeric actin (ASA). Nuclei were stained with DAPI. Scale bar: 50 μm.