Lab resource: Stem Cell Line

Generation of the Becker muscular dystrophy patient derived induced pluripotent stem cell line carrying the DMD splicing mutation c.1705-8 T>C.

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ABSTRACT

Becker Muscular dystrophy (BMD) is an X-linked syndrome characterized by progressive muscle weakness. BMD is generally less severe than Duchenne Muscular Dystrophy. BMD is caused by mutations in the dystrophin gene that normally give rise to the production of a truncated but partially functional dystrophin protein. We generated an induced pluripotent cell line from dermal fibroblasts of a BMD patient carrying a splice mutation in the dystrophin gene (c.1705-8 T>C). The iPSC cell-line displayed the characteristic pluripotent-like morphology, expressed pluripotency markers, differentiated into cells of the three germ layers and had a normal karyotype.

Resource Table:

| Unique stem cell line identifier | CCMi004-A |
|----------------------------------|----------|
| Alternative name(s) of stem cell line | BMD3 c.13 |
| Institution | Centro Cardiologico Monzino-IRCCS |
| Contact information of distributor | Aoife Gowran; aoife.gowran@ccfm.it |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | Age: 5 (at skin biopsy) |
| Clonality | Clonal |
| Cell Source | Dermal fibroblasts |
| Genetic Modification | YES |
| Type of Modification | Spontaneous mutation |
| Associated disease | Becker Muscular dystrophy |
| Gene/locus | DMD gene, Xp21.2-p21.1 |
| Method of modification | No modification |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | July 2019 |
| Cell line repository/bank | The Telethon Biobank and the Eurobiobank |

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The patient's fibroblasts were electroporated with plasmids encoding L-
function. DMD (Muscular Dystrophy) and will help the screening
process in BMD, from asymptomatic patients to those restricted to a
wheelchair by age sixteen. Although the majority of patients survive to
late adulthood (50–60 years), many develop cardiomyopathy char-
acterized by a progressive decline in ejection fraction and heart failure.

After obtaining informed consent and institutional ethical approval
dermal fibroblasts were isolated from a 5-year-old child with BMD. To
from CCMi004-A showed the presence of the single nucleotide sub-
Fig. 1E). Sanger sequencing, performed on genomic DNA extracted
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Materials and methods

Reprogramming of BMD patient's fibroblasts to iPSCs

The fibroblasts were isolated from the patient's skin biopsy by explant
culture. Fibroblasts were transfected with 1.25 µg of episomal vectors
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Flow cytometry

iPSCs detached using ReLeSR™ (Stemcell Technologies), were re-
suspended in PBS/0.5 mM EDTA, fixed for 20 min on ice using BD

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Karyotyping

Metaphase chromosomes were harvested from iPSC cultures at pas-
tivated in mFreSR™ (Stemcell Technologies) and stored at −180 °C for future experiments (Table 1).

Pluripotency marker immunocytochemistry

CCMi004-A were cultured in vitronectin-coated chamber slides for analysis of pluripotency proteins (SSEA4). iPSCs were fixed in 4% for-
maldehyde (10 min RT), treated with 0.1% Triton-X 100 in PBS for 5

In vitro trilineage differentiation potential assay

CCMi004-A cells were differentiated into cells of the ectodermal or

Karyotype analyses, performed on more than 30 metaphases, demonstrated that the iPSC line has a normal karyotype

In vitro trilineage differentiation assays showed that CCMi004-A is able to differentiate into cells of each germ layer (Ectoderm NESTIN/ PAX6, mesoderm cardiac troponin T type 2 CTNT2 and endoderm SOX17; Fig. 1D). Karyotype analyses, performed on more than 30 metaphases, demonstrated that the iPSC line has a normal karyotype

All antibody details are listed in Table 2.

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obtained at 100X magnification (Olympus BX microscope, U-CMAD3 Olympus camera). About 30 metaphases were analysed and karyotyped using an automated cytogenetic imaging system (MetaSystems Gmbh, Germany).

Dystrophin mutation analyses by Sanger sequencing

DNA was extracted from iPSCs using the QIAamp DNA Mini Kit (Qiagen) according to manufacturer's instructions. The DMD intron 14–15 was amplified with GoTaq Flexi DNA polymerase (Promega) using exon flanking primers (95 °C-56 °C-72 °C, 35 cycles). PCR products were then sent to Microsynth for direct Sanger sequencing. Electropherograms were aligned and analysed with ChromasPro software (Technelysium Pty Ltd). DNA extracted from iPSCs obtained from a healthy individual's dermal fibroblasts were used as controls.

STR analysis

STR analysis was performed by the ATCC cell-line authentication service. Seventeen STR loci plus the gender-determining locus,
Amelogenin, were amplified using the commercially available PowerPlex® 18D Kit from Promega. The cell-line sample was processed using the ABI Prism® 3500xl Genetic Analyzer. Data were analyzed using GeneMapper® ID-X v1.2 software (Applied Biosystems). Appropriate positive and negative controls were run and confirmed for each sample submitted.

**Mycoplasma analyses**

To verify the absence of Mycoplasma we used EZ-PCR Mycoplasma Detection Kit (Biological Industries) according to the manufacturer’s instructions. A positive control was included in the kit.

**Declaration of Competing Interest**

The authors declare that they are unaware of any conflict of interests associated with this work.

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**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2020.101819.

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