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Lab resource: Stem Cell Line

# Generation of a human induced pluripotent stem cell line, YCMi002-A, from a Factor VII deficiency patient carrying F7 mutations

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A B S T R A C T

Factor VII (FVII) deficiency is the most common among the rare bleeding disorders, which is caused by mutations in coagulation factor VII. Clinical features caused by FVII deficiency vary from mild or asymptomatic to fatal cerebral hemorrhage. We generated an induced pluripotent stem cell (iPSC) line, YCMi002-A, from FVII deficiency patient-derived fibroblasts. YCMi002-A cells are characterized by novel compound heterozygous mutations. The c.345C > A; p.C115X is well known and the second one, c.1276C > T; p.Q426X, remains novel. YCMi002-A cells may help researchers to understand correlation between these mutations and the symptoms of FVII deficiency.

## 1. Resource Table

| | |
|---|---|
| Unique stem cell line identifier | YCMi002-A |
| Alternative name(s) of stem cell line | F7epi7 |
| Institution | Yonsei University, College of Medicine, Seoul, Korea |
| Contact information of distributor | Dong-Wook Kim, dwkim2@yuhs.ac |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | Age: 19<br>Sex: Female<br>Ethnicity: Korean |
| Cell Source | Fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Transgene free (episomal plasmid) |
| Genetic Modification | YES |
| Type of Modification | Hereditary |
| Associated disease | Factor VII Deficiency |
| Gene/locus | F7 (NM_000131.4) - c.345C > A, c.1276C > T / 13q34 |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | Feb. 2020 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/YCMi002-A |
| Ethical approval | Ethical committee: Yonsei university health system, Severance Hospital, Institutional review board approval number: #4–2012-0028 |

## 2. Resource utility

Novel iPSCs were derived from an FVII compound heterozygous patient with two mutations, one well-known and one novel. These iPSCs may be helpful to study the correlation between the severity of FVII deficiency and mutations.

## 3. Resource details

Factor VII (FVII) deficiency is the most frequent among rare bleeding disorders. It is usually transmitted in an autosomal recessive manner with an incidence of one per 500,000 in the general population (Perry, 2002). Clinical symptoms are heterogeneous and range from miscellaneous minor bleeding to severe life-threatening hemorrhages, such as cerebral, gastrointestinal, and joint hemorrhages (Lapecorella et al., 2008). Various types of mutations are responsible for FVII deficiency, however, most of them are point mutations (The F7 mutations database, http://www.umd.be/F7/W_F7/index.html). In this study, we generated an induced pluripotent stem cell (iPSC) line, YCMi002-A, from a female patient carrying a novel nonsense mutation. We reprogrammed patient-derived fibroblasts into iPSCs using integration-free

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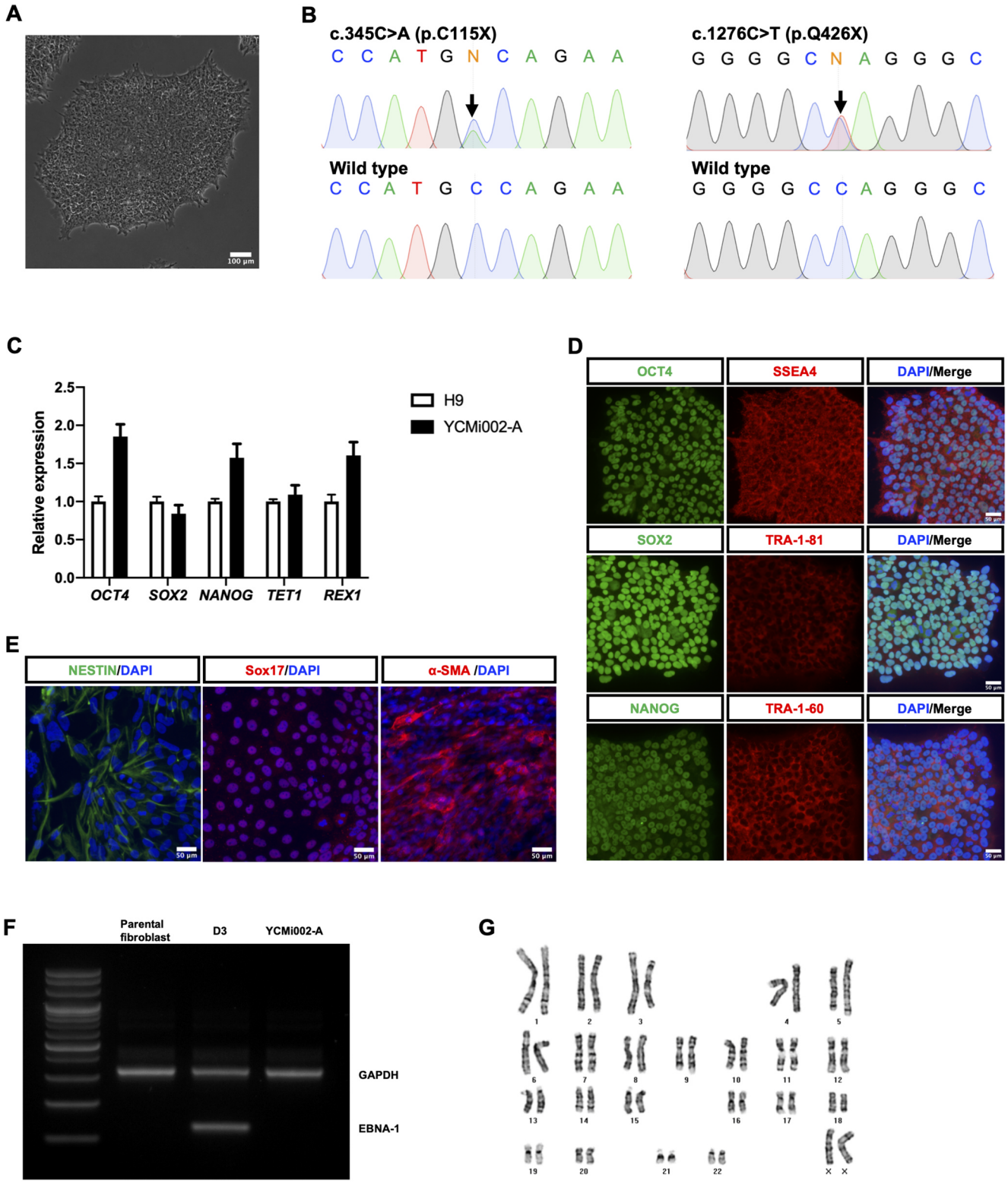


**Fig. 1.** Characterization of YCMi002-A iPSC line.

episomal plasmids expressing five pluripotency factors (OCT4, SOX2, KLF4, L-MYC, and Lin28). After the induction of iPSC, we picked several iPSC colonies with a normal human embryonic stem-cell (hESC) like morphology (Fig. 1A). After selection, we checked the expressions of several pluripotency markers by immunostaining. These colonies highly expressed the pluripotency markers (OCT4, SOX2, and NANOG), and the surface markers (SSEA4, TRA-1-81, and TRA-1-60) (Fig. 1D). The expression level of pluripotency markers measured using quantitative-polymerase chain reactions (PCR) in YCMi002-A was similar to human embryonic stem cell H9 (Fig. 1C). Pluripotency was further evaluated by differentiation of three germ layers. Using embryoid body (EB) formation assay, we demonstrated that YCMi002-A cells could be differentiated into the principal cells in three germ layers with expression of ectodermal marker Nestin, endodermal marker Sox17, and

mesodermal marker α-SMA (Fig. 1E). We identified heterozygous compound mutations in YCMi002-A by Sanger sequencing (Fig. 1B). The first mutation is a well-known mutant located in exon 5 of Factor VII (c.345C > A; p.C115X), and the other is novel and located in exon 9 (c.1276C > T; p.Q426X). We checked for remaining episomal plasmids in YCMi002-A using PCR with episomal plasmid-specific primers. We did not detect the episomal plasmids after passage 16 or insertion into established YCMi002-A (Fig. 1F, Day 3 (D3) for positive control). YCMi002-A cells showed a normal 46, XX karyotype after 16 passages in culture (Fig. 1G). Short tandem repeat (STR) analysis was performed on genomic DNA extracted from YCMi002-A cells and parental fibroblasts. We found an exact match in all the 18 STR loci analyzed, which shows that the cell line was not contaminated by any other human cell lines and genetically identical with parental fibroblasts (submitted in archive with journal). YCMi002-A cells were shown to be free of mycoplasma contamination (supplementary data).

## 4. Materials and methods

### 4.1. Cell culture & generation of iPSCs

Episomal plasmids encoding reprogramming factors were used as reported (Okita et al., 2011). In brief, patient-derived fibroblasts grown in DMEM supplemented with 10% FBS and 1% NEAA were electroporated using a microporator system (Neon, Thermo Fisher Scientific) with 3 μg of episomal plasmid mixtures. After three 10 ms pulse with a voltage of 1,650, the cells were grown in DMEM containing 10% FBS and 1% NEAA. Seven days after transfection, cells were transferred onto a STO feeder layer. iPSC colonies like hESCs were picked up mechanically and further cultured for characterization (Table 1).

### 4.2. RNA extraction, cDNA synthesis, and qPCR

Total RNA was extracted using the Easy-Spin® Total RNA Extraction kit (iNtRON Biotechnology) according to the manufacturer's instructions. cDNA was synthesized from 1 μg of total RNA using PrimeScript™ RT Master Mix (TAKARA Bio Inc.). qPCR was performed using FastFire qPCR PreMix (TIANGEN) in a CFX96 Real-Time System (Bio-Rad). The transcription level for each targeted gene was normalized to GAPDH expression. Primer sequences are listed in Table 2.

### 4.3. PCR

Total genomic DNA was extracted from YCMi002-A cells and parental fibroblasts by DNeasy Blood & Tissue kit (Qiagen). PCR was performed by a PCR thermal cycler (Applied Biosystems Geneamp 2720) using EmeraldAmp® GT PCR Master Mix (TAKARA Bio Inc.) according to the manufacturer's instructions.

### 4.4. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 15 min at room temperature. After permeabilization with 0.1% Triton X-100 in PBS, cells were blocked with 2% BSA-PBS for 1 h at room temperature, and incubated overnight at 4 °C with primary antibodies in 2% BSA-PBS (Table 2). Cell were then washed, labeled with fluorescence-tagged secondary antibodies (Thermo Fisher Scientific) for 30 min at room temperature and mounted in DAPI mounting medium (Vector Laboratories). Images were obtained with an Olympus IX71 microscope equipped with a DP71 digital camera (Olympus FSX100 system).

### 4.5. Differentiation into three germ layers

To test the differentiation of YCMi002-A cells *in vitro*, embryoid bodies (EBs) were formed by partial dissociation and cultured in DMEM/F12 (1:1) medium supplemented with 20% knockout serum (Invitrogen), 4.5 g/L L-glutamine, 1% NEAA, 0.1 mM 2-mercaptoethanol, and 15% FBS. 7 days after induction, EBs were attached onto Matrigel-coated culture dishes and further cultured for 14 days. Spontaneous differentiation of EBs into the three germ layer lineages was detected by immunostaining with appropriate antibodies (Table 2).

### 4.6. Karyotyping and mycoplasma testing

G-banding karyotype analysis was performed at passage 16 at GenDix, Inc. using standard protocols for GTG banding. Total 20 metaphases were analyzed at 550 band resolution. The absence of mycoplasma contamination was tested by e-Myco™ Mycoplasma PCR Detection Kit (iNtRON Biotechnology).

### 4.7. STR analysis

STR assay was performed by Cosmogenetech co, Ltd. (Korea). Briefly, the target loci were amplified using PowerPlex® 18D System Kit (Promega) and analyzed with ABI3130xl genetic analyzer (Applied Biosystems) using the software program GeneMapper v.5.0 (Applied Biosystems).

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | Normal | Fig. 1 panel A |
| Phenotype | Qualitative analysis Immunocytochemistry | Positive for pluripotency markers including OCT4, SOX2, NANOG, SSEA4, TRA-1-81, and TRA-1-60 | Fig. 1 panel D |
| | Quantitative analysis RT-qPCR | Positive for pluripotency markers including OCT4, SOX2, NANOG, TET1, REX1 | Fig. 1 panel C |
| Genotype | Karyotype (G-banding) and resolution | 46, XX Resolution 550 | Fig. 1 panel G |
| Identity | Microsatellite PCR (mPCR) OR | N/A | |
| | STR analysis | 18 sites were tested and all matched | submitted in archive with journal |
| Mutation analysis (IF APPLICABLE) | Sequencing | Compound Heterozygote | Fig. 1 panel B |
| | Southern Blot OR WGS | N/A | |
| Microbiology and virology | Mycoplasma | Mycoplasma testing via PCR. Negative | supplementary |
| Differentiation potential | Embryoid body formation | NESTIN (ectoderm); Sox17 (endoderm); α-SMA (mesoderm) | Fig. 1 panel E |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A | |
| Genotype additional info (OPTIONAL) | Blood group genotyping | N/A | |
| | HLA tissue typing | N/A | |

**Table 2**
Reagents details.

| Antibodies used for immunocytochemistry/flow-citometry | | | |
|---|---|---|---|
| | Antibody | Dilution | Company Cat # and RRID |
| Pluripotency markers | Rabbit anti-OCT4 | 1:200 | Santa Cruz, cat #sc-9081, RRID:AB_2167703 |
| | Rabbit anti-SOX2 | 1:200 | Millipore, cat # AB5603 RRID:AB_304980 |
| | Goat anti-NANOG | 1:50 | R&D systems, cat #AF1997 RRID:AB_355097 |
| | Mouse anti-SSEA4 | 1:200 | Millipore, cat #MAB4304 RRID:AB_177629 |
| | Mouse anti-TRA-1-81 | 1:100 | Millipore, cat #MAB4381 RRID:AB_177638 |
| | Mouse anti-TRA-1-60 | 1:100 | Millipore, cat #MAB4360 RRID:AB_2277963 |
| Differentiation markers | Rabbit anti-NESTIN | 1:1000 | Millipore, cat #ABD69 RRID:AB_2744681 |
| | Goat anti-SOX17 | 1:200 | Santa Cruz, cat#sc-17356 RRID:AB_2195655 |
| | Mouse anti-α-SMA | 1:400 | Sigma-Aldrich, cat #A5228 RRID:AB_262054 |
| Secondary antibodies | Donkey anti-rabbit IgG (H + L) highly cross-adsorbed secondary antibody, Alexa Fluor 488 | 1:1000 | Invitrogen, cat #A-21206 RRID:AB_2535792 |
| | Donkey anti-goat IgG (H + L) cross-adsorbed secondary antibody, Alexa Fluor 488 | 1:1000 | Invitrogen, cat #A-11055 RRID:AB_2534102 |
| | Donkey anti-mouse IgG (H + L) highly cross-adsorbed secondary antibody, Alexa Fluor 488 | 1:1000 | Invitrogen, cat #A-21202 RRID:AB_141607 |
| | Donkey anti-mouse IgG (H + L) highly cross-adsorbed secondary antibody, Alexa Fluor 594 | 1:1000 | Invitrogen, cat #A-21203 RRID:AB_141633 |
| | Donkey anti-goat IgG (H + L) cross-adsorbed secondary antibody, Alexa Fluor 594 | 1:1000 | Invitrogen, cat #A-11058 RRID:AB_2534105 |
| | Donkey anti-rabbit IgG (H + L) highly cross-adsorbed secondary antibody, Alexa Fluor 594 | 1:1000 | Invitrogen, cat #A-21207 RRID:AB_141637 |
| **Primers** | | | |
| | **Target** | **Forward/Reverse primer (5′-3′)** | |
| Episomal vector (PCR) | EBNA-1 (128 bp) | ATGGACGAGGACGGGGAAGA/ GCCAATGCAACTTGGACGTT | |
| Pluripotency markers (qPCR) | OCT4 (164 bp) | CCTCACTTCACTGCACTGTA / CAGGTTTTCTTTCCCTAGCT | |
| | SOX2 (80 bp) | TTCACATGTCCCAGCACTACCAGA / TCACATGTGTGAGAGGGGCAGTGTGC | |
| | NANOG (154 bp) | TGAACCTCAGCTACAAACAG / TGGTGGTAGGAAGAGTAAAG | |
| | TET1 (165 bp) | CTGCAGCTGTCTTGATCGAGTTAT / CCTTCTTTACCGGTGTACACTACT | |
| | REX1 (145 bp) | TCACAGTCCAGCAGGTGTTTG / TCTTGTCTTTGCCCGTTTCT | |
| House-Keeping Genes (PCR) | GAPDH (327 bp) | GAACATCATCCCTGCCTCTACTG/ CAGGAAATGAGCTTGACAAAGTGG | |
| House-Keeping Genes (qPCR) | GAPDH (159 bp) | CAATGACCCCTTCATTGACC / TTGATTTTGGAGGGATCTCG | |

## 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.

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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.2020.102026.

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