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# Generation and characterization of induced pluripotent stem cells from breast cancer patients carrying ATM mutations

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## Abstract

We generated two induced pluripotent stem cell (iPSC) lines from peripheral blood mononuclear cells (PBMCs) of breast cancer patients carrying germline ATM mutations, a gene associated with a 7% prevalence in breast cancer. These iPSC lines displayed typical morphology, expressed pluripotency markers, maintained a stable karyotype, and retained the ability to differentiate into the three germ layers. These patient-specific iPSC lines hold great potential for mechanistic investigations and the development of drug screening strategies aimed at addressing ATM-related cancer.

**Resource Table:**

| | |
|---|---|
| Unique stem cell lines identifier | 1. SCVIi083-A<br>2. SCVIi084-A |
| Alternative name(s) of stem cell lines | 1. SCVI2655 (SCVIi083-A) |
| | 2. SCVI2591 (SCVIi084-A) |
| Institution | Stanford Cardiovascular Institute, Stanford, CA, US |
| Contact information of distributor | Joseph C. Wu, joewu@stanford.edu |
| Type of cell lines | iPSC |
| Origin | Human |
| Additional origin info required | Age: 72 (SCVIi083-A) and 23 (SCVIi084-A) |



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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J. C.W. is a co-founder of Greenstone Biosciences. 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.

| | |
|---|---|
| *for human ESC or iPSC* | Sex: Female Ethnicity if known: White (SCVIi083-A); East Asian (SCVIi084-A) |
| Cell Source | PBMCs |
| Clonality | Clonal |
| Method of reprogramming | Nonintegrating Sendai virus expression of human OCT4, SOX2, KLF4, and c-MYC |
| Genetic Modification | YES |
| Type of Genetic Modification | Spontaneous/naturally occurred mutation |
| Evidence of the reprogramming transgene loss (including genomic copy if applicable) | *RT-/q-PCR* |
| Associated disease | Breast Cancer |
| Gene/locus | Chromosome location: 11q22.3<br>SCVIi083-A: GRCh38: 11:108289006–108289007<br>SCVIi084-A: GRCh38: 11:108307919–108307919 |
| Date archived/stock date | SCVIi083-A: 07/07/2022; SCVIi084-A: 05/25/2022 |
| Cell line repository/bank | https://hpscreg.eu/cell-line/SCVIi083-A<br>https://hpscreg.eu/cell-line/SCVIi084-A |
| Ethical approval | The generation of these iPSC lines was approved by the Administrative Panel on Human Subjects Research under Institutional Review Board (IRB) #29904 “Derivation of Human Induced Pluripotent Stem Cells (Biorepository)” |

## 1. Resource utility

Germline mutations in the ATM gene are known to cause the autosomal recessive Ataxia Telangiectasia disease and also confer an increased risk of developing breast cancer. Generating iPSC lines carrying ATM variants provide an unlimited source for disease modeling, gene therapy, and screening of compounds for potential therapeutic effects (Table 1).

## 2. Resource details

The Ataxia Telangiectasia Mutated (ATM) gene, also known as ATM serine/threonine kinase, is a crucial tumor suppressor gene that belongs to the phosphatidylinositol 3-kinase-related protein kinase (PIKK) superfamily. It plays a pivotal role in DNA repair and cell cycle control (Moslemi et al., 2021). Mutations in the ATM gene are the primary cause of Ataxia Telangiectasia, an autosomal recessive neurodegenerative disorder. Recent studies have revealed a significant association between ATM variants and the risk of multiple types of cancers, particularly breast cancer (Stucci et al., 2021). Carriers of pathogenic ATM variants have a 2 to 4-fold increased risk of developing breast cancer (McDuff et al., 2021), especially early-onset cancer and bilateral breast cancer (Renwick et al., 2006). To understand these genetic associations, we successfully generated and characterized iPSC lines derived from female donors carrying specific ATM variants: c.4143dup and c.5697C > A, respectively. These iPSC lines serve as renewable and genetically relevant cellular models for investigating disease pathology and conducting drug screening for precision medicine.

We recruited two breast cancer patients, a 72-year-old White female who developed breast cancer, stage II (T2N1M0), of the left breast and a 23-year-old East Asian female who developed breast cancer, stage IV (T3N3M1), of the left breast. Genetic testing demonstrated that they carried the pathogenic variants *ATM* c.4143dup (ClinVar ID: 181880) and c.5697C > A (ClinVar ID: 421488), respectively. Using a Sendai Virus-based vector carrying the Yamanaka factors *OCT4, SOX2, KLF4*, and *c-MYC* (Mondéjar-Parreño et al., 2021), we successfully generated iPSCs from the patients' peripheral blood mononuclear cells (PBMCs), named SCVIi083-A and SCVIi084-A. Both lines exhibited typical stem cell morphology when observed under bright field microscope (Fig. 1A). These cells expressed pluripotent markers (SOX2, NANOG, and POU5F1) and lost the expression of Sendai virus vector (SEV), as shown by quantitative RT-PCR (Fig. 1C). The iPSCs were further analyzed for pluripotency markers using immunofluorescence staining (Fig. 1B). The iPSC lines tested negative for mycoplasma (Fig. 1D). Karyotype analysis exhibited normal female chromosomes (Fig. 1E). Sanger sequencing demonstrated a heterozygous mutation of c.4143dup in SCVIi083-A and c.5697(C > A) in SCVIi084-A (Fig. 1F). Short tandem repeat (STR) analysis of the parental PBMCs and derived iPSCs confirmed clonal identity (Submitted in the archive with journal). Both lines were successfully differentiated into the three germ layers (Fig. 1G).

## 3. Materials and methods

### 3.1. Generation of human induced pluripotent stem cells (iPSCs)

PBMCs were isolated from the patient's whole blood samples by Percoll gradient separation, purified by multiple washes in DPBS, and cultured in the StemPro®−34 SFM medium (100 ng/mL SCF, 100 ng/ mL FLT3, 20 ng/mL IL-3, 20 ng/mL IL-6, and 20 ng/mL EPO (ThermoFisher Scientific). The CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher Scientific) was used for reprogramming the PBMCs following the manufacturer's instructions.

### 3.2. Cell culture

The iPSCs were passaged at 90 % confluency using Gentle Cell Dissociation Reagent (STEMCELL™ technologies). Detached cells were resuspended in Brew medium with 5 μM ROCK1 inhibitor (SelleckChem), and replated onto Matrigel-coated (1:500) 6-well plates. Cells were cultured for 24 hr at 37 °C with 5 % $CO_2$, and after that the media was replaced with Brew medium every two days.

### 3.3. RNA extraction and RT-qPCR

Total RNA was extracted from iPSCs at passage 13 using the miRNeasy Micro Kit (Qiagen), then cDNA was synthesized using the iScript™ Reverse Transcription Supermix (BIO-RAD). Target genes were examined using the TagMan™ Universal PCR Master Mix (ThermoFisher Scientific) and probes (Table 2).

### 3.4. Immunofluorescence staining

Cells at passage 18 were fixed with 4 % paraformaldehyde for 20 min. After two washes with DPBS, the cells were permeabilized using 0.1 % Triton X 100 in DPBS for 10 min.

Subsequently, blocking was performed with 10 % goat serum in DPBS for 1 hr. Cells were then incubated with primary antibodies (Table 2) overnight at 4 °C. On day 2, the cells were washed with DPBS and incubated with the corresponding secondary antibodies (Table 2) for 1 hr at room temperature. The nuclei were counterstained with NucBlue Probes (ThermoFisher Scientific).

### 3.5. Karyotyping

The Karyotyping was performed on iPSCs at passage 12 using the KaryoStat™ assay (ThermoFisher Scientific).

### 3.6. Targeted sequencing

The genomic DNA was extracted using the QuickExtract™ DNA extraction solution (Biosearch Technologies). The PCR assay was performed using the PrimeSTAR GXL DNA Polymerase (Clontech) and the primers (Table 2) under the following conditions: 98 °C for 5 s 60 °C for 15 s, 72 °C for 30 s for 35 cycles. The PCR products were purified, and the sequencing was performed at Stanford Protein and Nuclear Acid (PAN) facility.

### 3.7. Mycoplasma detection

The mycoplasma test was conducted on iPSCs at passage 13 using the MycoAlertTM Detection Kit (Lonza), following the manufacturer's instructions.

### 3.8. Trilineage differentiation

iPSCs at passage 17 were used for all three-germ layer differentiation. The StemXVivo Ectoderm kit (R & D systems) and the StemDiff™ Definitive Endoderm differentiation kit (STEMCELL™ Technologies) were used to derive the ectoderm and the endoderm, respectively. Mesoderm differentiation was induced by 6 μM CHIR-99021 (Selleck Chemicals) in RPMI media supplemented with B27 minus Insulin for 48 hr.

### 3.9. Short tandem repeat (STR) analysis

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen). PCR and capillary electrophoresis were performed using the CLA IdentiFiler™ Direct PCR Amplification Kit and ABI3130xl at the Stanford PAN facility.

## Acknowledgments

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## Data availability

Data will be made available on request.

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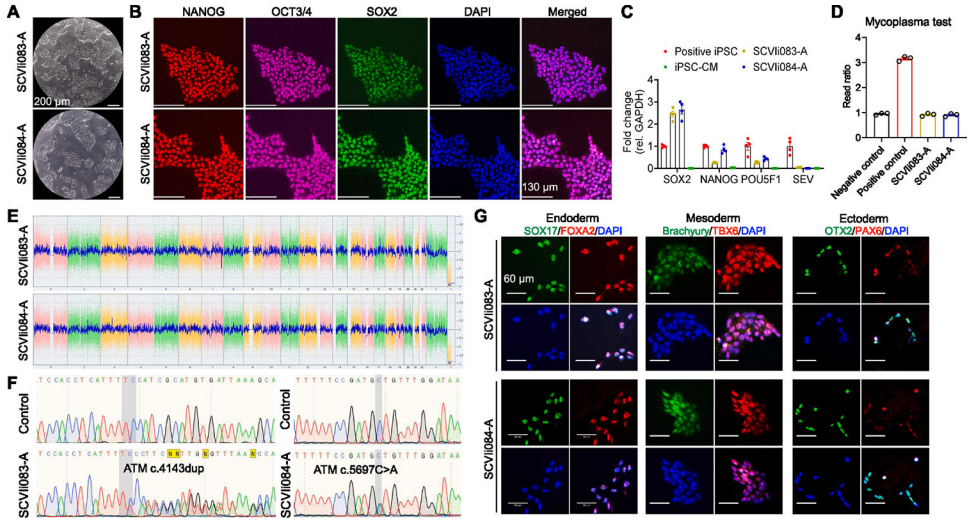


**Fig. 1.**

Characterization of two iPSC lines derived from breast cancer patients carrying ATM mutations.

**Table 1**

Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography brightfield | Normal | Fig. 1 panel A |
| Phenotype | Qualitative analysis: Immunofluorescence staining | Positive expression of pluripotency markers: Oct3/4, Nanog, Sox2 | Fig. 1 panel B |
| | Quantitative analysis: RT-qPCR | High expression levels of pluripotency markers (Nanog, Sox2, and Pou5f1) in iPSCs but significantly reduced in iPSC-CMs | Fig. 1 panel C |
| Genotype | Karyotype (G-banding) and resolution | Karyostat™ Assay, resolution 1–2 Mb: Normal karyotype: 46, XX | Fig. 1 panel E |
| Identity | Microsatellite PCR (mPCR) or | DNA profiling not performed | N/A |
| | STR analysis | 16 loci tested, matched | Submitted in archive with journal |
| Mutation analysis (IF APPLICABLE) | Sequencing | Heterozygous<br>SCVIi083-A: c.4143dup<br>SCVIi084-A: c.5697C > A | Fig. 1 panel F |
| | Southern Blot OR WGS | N/A | N/A |
| Microbiology and virology | Mycoplasma test: Luminescence | Negative | Fig. 1 panel D |
| Differentiation potential | Directed differentiation of the three germ layers | Positive staining for all three germ layer markers | Fig. 1 panel G |
| List of recommended germ layer markers | Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer | Positive expression of germ layer markers:<br>Ectoderm: Pax6, Otx2;<br>Endoderm: Sox17, Foxa2;<br>Mesoderm: Brachyury, Tbx6 | Fig. 1 panel G |
| Donor screening (OPTIONAL) | HIV 1 + 2 Hepatitis B, Hepatitis C | *N/A* | *N/A* |
| Genotype additional info (OPTIONAL) | Blood group genotyping | *N/A* | *N/A* |
| | HLA tissue typing | *N/A* | *N/A* |

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**Table 2**

Reagents details.

| | **Antibodies used for immunocytochemistry/flow-cytometry** | | | |
|---|---|---|---|---|
| | **Antibody** | **Dilution** | **Company Cat #** | **RRID** |
| *Pluripotency Markers* | Rabbit Anti-Nanog | 1:100 | Proteintech Cat #14295–1-AP | RRID: AB_1607719 |
| | Mouse $IgG_{2b}$ κOct3/4 antibody | 1:100 | Santa Cruz Biotechnology Cat #sc-5279 | RRID: AB_628051 |
| | Goat IgG anti-Sox2 | 1:100 | R and D Systems Cat #AF2018 | RRID: AB_355110 |
| *Ectoderm Markers* | Goat Anti-Otx2 | 1:200 | R and D Systems Cat #AF1979 | RRID: AB_2157172 |
| | Rabbit Anti-Pax6 | 1:200 | Thermo Fisher Scientific Cat #42–6600 | RRID: AB_2533534 |
| *Endoderm Markers* | Goat Anti-Sox17 | 1:200 | R and D Systems Cat #AF1924 | RRID: AB_355060 |
| | Rabbit Anti-Foxa2 | 1:250 | Thermo Fisher Scientific Cat #701698 | RRID: AB_2576439 |
| *Mesoderm Markers* | Goat Anti-Brachyury | 1:200 | R and D Systems Cat #AF2085 | RRID: AB_2200235 |
| | Rabbit Anti-Tbx6 | 1:200 | Thermo Fisher Scientific Cat #PA5–35102 | RRID: AB_2552412 |
| *Secondary antibodies* | Alexa Fluor 647 Goat Anti-Mouse $IgG_{2b}$ | 1:250 | Thermo Fisher Scientific Cat #A-21242 | RRID: AB_2535811 |
| | Alexa Fluor 555 Goat Anti-Rabbit IgG (H + L) | 1:500 | Thermo Fisher Scientific Cat #A-21428 | RRID: AB_141784 |
| | Alexa Fluor 488 Donkey Anti-Goat IgG | 1:1000 | Thermo Fisher Scientific Cat #A-11055 | RRID: AB_2534102 |
| | **Primers Target** | **Size of band** | **Forward/Reverse primer (5'-3')** | |
| *Sendai virus plasmid (qPCR)* | Sendai virus genome | 181 bp | Mr042698800_mr (Thermo Fisher Scientific) | |
| *Pluripotency Markers (qPCR)* | NANOG | 109 bp | Hs02387400_g1 (Thermo Fisher Scientific) | |
| *House-Keeping Genes (qPCR)* | POU5F1 | 77 bp | Hs00999632_g1 (Thermo Fisher Scientific) | |
| *Pluripotency Markers (qPCR)* | SOX2 | 86 bp | Hs04234836_s1 (Thermo Fisher Scientific) | |
| *House-Keeping Genes (qPCR)* | GAPDH | 157 bp | Hs02786624_g1 (Thermo Fisher Scientific) | |
| *Genotyping* | ATM c.4143dup | 303 bp | Forward: 5'- TGGAAGTTCACTGGTCTA -3' Reverse: 5'- GTGTCACAAGATTCTGTTC - 3' | |

| Antibodies used for immunocytochemistry/flow-cytometry | | | | |
|---|---|---|---|---|
| **Antibody** | | **Dilution** | **Company Cat #** | **RRID** |
| *Genotyping* | ATM c.5697C > A | 656 bp | Forward: 5'- CCACCAGAACCTTATAGC - 3'<br>Reverse: 5'- CACAGTATTGCCACAGAT - 3' | |