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Amandine Caillaud, Lise Bray, Aurore Girardeau, Zoé Begué-Racapé, Lucie Vince, et al.. Generation of an induced pluripotent stem cell line (ITXi012-A) from a patient with genetically determined high-lipoprotein(a) plasma levels. *Stem Cell Research*, 2023, 72, 10.1016/j.scr.2023.103205 . hal-04212298

HAL Id: hal-04212298

<https://hal.science/hal-04212298>

Submitted on 20 Sep 2023

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Lab Resource: Single Cell Line



Generation of an induced pluripotent stem cell line (ITXi012-A) from a patient with genetically determined high-lipoprotein(a) plasma levels

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ABSTRACT

Elevated circulating lipoprotein(a) (Lp(a)) is a genetically determined risk factor for coronary artery disease and aortic valve stenosis (Tsimikas, 2017). Importantly, the LPA gene, which encodes the apolipoprotein(a) (protein-component of Lp(a)), is missing in most species, and human liver cell-lines do not secrete Lp(a). There is a need for the development of human in vitro models suitable for investigating biological mechanisms involved in Lp(a) metabolism. We here generated and characterized iPSCs from a patient with extremely high Lp(a) plasma levels genetically determined (Coassin et al., 2022). This unique cellular model offers great opportunities and new perspectives for investigations on biological mechanisms involved in Lp(a) metabolism.

1. Resource Table

Unique stem cell line identifier	ITXi012-A
Alternative name(s) of stem cell line	Lp(a) Clone 23
Institution	L'institut du thorax Inserm UMR 1087/CNRS UMR 6291 44007 Nantes cedex 1, France
Contact information of distributor	Bertrand Cariou bertrand.cariou@univ-nantes.fr
Type of cell line	Induced pluripotent stem cells (iPSCs)
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 28 Sex: Male
Cell Source	Urine progenitor cells
Clonality	Clonal
Method of reprogramming	Integration-free episomal expression of Oct4, Sox2, Lin28, Klf4 et L-Myc (Epi5™ Episomal iPSC reprogramming kit, Cat#A15960).
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR on agarose gel electrophoresis
Associated disease	Extremely high Lp(a) plasma levels, coronary artery disease
Gene/locus	ITXi012-A-cells are derived from a patient previously described by Coassin et al. (Coassin et al., 2022) (patient III_C4 in a large familial pedigree), who carries

(continued on next column)

(continued)

Date archived/stock date	2020-05-04
Cell line repository/bank	https://hpscereg.eu/cell-line/ITXi012-A
Ethical approval	Authorization from the French Ministry of Health (ID:DC-2011-1399)

2. Resource utility

To our knowledge, the ITXi012-A cell-line is the first hiPSC-line generated from a patient with genetically determined high Lp(a) plasma levels (Coassin et al., 2022). Upon differentiation towards hepatocyte-like cells (Si-Tayeb et al., 2015), this cell-line will provide a new model for the study of Lp(a) metabolism. A detailed characterization of ITXi012-A has been performed and related data is summarized in Table 1 and Table 2.

3. Resource details

In the present study, urine progenitor cells from a 28-year-old male patient carrying extremely high Lp(a) plasma levels, were reprogrammed using episomal vectors. The reprogrammed ITXi012-A cells

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<https://doi.org/10.1016/j.scr.2023.103205>

Received 17 April 2023; Received in revised form 11 September 2023; Accepted 13 September 2023

Available online 15 September 2023

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present with classical hiPSC-like morphology (Fig. 1A), express pluripotent markers at the transcriptional level (*markers: POU5F1, NANOG* and *SOX2*, Fig. 1B) and at the protein level as shown by immunostaining (markers: TRA1-60 and OCT3/4, Fig. 1C). The proportion of cells expressing pluripotency markers (SSEA4, TRA1-60) was assessed by Fluorescence-activated cell sorting (with SSEA4: 98,4% and TRA-60: 73,2% positive cells) (Fig. 1D). ITXi012-A cells have the capacity to differentiate into three germ layers, as confirmed in vitro by measuring the expression of specific: endoderm (*FOXA2*), mesoderm (*HAND1*) and ectoderm (*PAX6*) markers (Fig. 1E). Importantly, we demonstrate that ITXi012-A cells come from the urine progenitor cells they were originally reprogrammed from (paternity test of 16 STR) (STR analysis, Archived). We further confirm by PCR, that ITXi012-A, at passage 16, do not express episomal marks used for reprogramming anymore (Fig. 1F). ITXi012-A cells do not present with genomic integrity alterations (analysis of 24 copy number variations and G-banding staining) (Supplementary file 1) and are mycoplasma free (Fig. 1G).

Overall, we provide circumstantial evidences showing that the ITXi012-A line harbors all characteristics needed for further differentiation and investigations of *Lp(a)*-related molecular mechanisms.

4. Materials and methods

4.1. Generation and maintenance of hiPSCs

3×10^5 urine progenitor cells were reprogrammed in TeSRTM E7TM medium (STEMCELLTM Technologies) using the Epi5TM Episomal iPSC Reprogramming kit (InvitrogenTM) and the Basic epithelial Nucleofector Kit (Lonza) with T013 program (Amaxa nucleofector). iPSC colonies were manually picked and transferred onto mitomycin-treated mouse-embryonic fibroblasts (MEFs) for amplification. At passage 5, ITXi0012-

A colonies were dissected from MEFs and transferred into feeder-free culture conditions. iPSCs were grown in hypoxic conditions in Stem-MACSTM iPS-Brew XF medium (Miltenyi Biotec) on 0,05 mg/mL Matrigel® and passaged at 80% confluency in a 1:3 ratio using the Gentle Cell Dissociation Reagent (STEMCELLTM Technologies).

4.2. Episomal marks analysis

Genomic DNA over 3 passages was isolated using the NucleoSpin Tissue Purification Kit (MACHEREY-NAGEL). The DNA was amplified by PCR using 50 ng of genomic DNA using dedicated primers for episomal vectors (Table 2). Epi5 kit-containing plasmids were used as positive controls and ITXi001-A cell line was used as negative control. PCR products were visualized on a 1,5% agarose gel.

4.3. Karyotyping

Genome stability was verified using both G-banding analysis and digital PCR covering the most recurrent abnormalities (Assou et al., 2020) (Duo iCS-Karyo service, Stemgenomics).

4.4. Mycoplasma testing

Culture supernatant of highly confluent cells (3 passages) was heat at 98 °C for 10 min. Mycoplasma DNA was amplified by PCR using 5 µl of supernatant (Table 2). A cell supernatant positive for mycoplasma was used as positive control. PCR products were visualized on a 1,5% agarose gel.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field image	Normal	Fig. 1A, Scale bar = 100 µm
Phenotype	Quantitative analysis (RT-qPCR, Flow cytometry)	RT-qPCR: expression of <i>NANOG</i> , <i>POU5F1</i> and <i>SOX2</i> Flow cytometry: TRA 1-60:73,2%, SSEA-4: 98,4%	Fig. 1B, Fig. 1D
	Qualitative analysis (Immunocytochemistry)	Expression of pluripotency markers: OCT3/4, TRA1-60	Fig. 1C, Scale bar = 100 µm
Genotype	G-banding karyotype and digital PCR (Stemgenomics)	Normal with chromosomal formula: 46, XY	Supplementary file 1
Identity	Microsatellite PCR (mPCR) OR STR analysis	not performed PCR single locus technology: 16 sites tested; all matched (100%)	N/A Archived
Mutation analysis (IF APPLICABLE)	Sequencing Southern Blot OR WGS	N/A e.g. number of insertions in genome, off-target effects	(Coassin et al., 2022) N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by PCR.	Fig. 1G
Differentiation potential	Directed differentiation	RT-qPCR analysis positive for <i>FOXA2</i> (endoderm), <i>HAND1</i> (mesoderm), <i>PAX6</i> (ectoderm)	Fig. 1E
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR)	Ectoderm: <i>PAX6</i> , Endoderm: <i>FOXA2</i> Mesoderm: <i>HAND1</i>	Fig. 1E
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

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency marker flow cytometry	REA CTRL PE	1/50	Miltenyi Biotec Cat# 130-113-438	RRID: AB_2733893
	anti-human SSEA-4 PE	1/11	Miltenyi Biotec Cat# 130-098-369	RRID: AB_2653519
	anti-human TRA1-60 PE	1/11	Miltenyi Biotec Cat# 130-100-347	RRID: AB_2654227
Pluripotency marker IF	Rat anti-Human OCT3/4	1/500	ebiosciences Cat# 14-5841-82	RRID: AB_914301
	Mouse anti-Human TRA1-60	1/500	ebiosciences Cat# 14-8863-82	RRID: AB_891610
Secondary antibodies	Goat anti-Rat 488 nm	1/1000	Thermo Fisher Scientific Cat# A-11006	RRID: AB_2534074
	Goat anti-mouse 488 nm	1/1000	Thermo Fisher Scientific Cat# A-11001	RRID: AB_2534069
Nuclear Stain	DAPI	10 µg/mL at 1/2000	Sigma cat # D9542	RRID: AB_24894305
Primers				
	Target	Size of band	Forward/Reverse primer (5'-3')	
Episomal Plasmid (PCR)	oriP (pEP4-SF1-oriP/pEP4-SR1-ori)	544 bp	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGACAAAC	
	EBNA-1 (pEP4-SF2-oriP/pEP4-SF2-oriP)	666 bp	ATCGTCAAAGCTGCACACAG/CCCAGGAGTCCCAGTAGTCA	
Differentiation Markers (qPCR)	PAX6	86 bp	Thermo Fisher Cat# Hs01088114-m1 (TaqMan®probe ID)	
	FOXA2	66 bp	Thermo Fisher Cat# Hs00232764-m1 (TaqMan®probe ID)	
	HAND1	65 bp	Thermo Fisher Cat# Hs02330376_s1 (TaqMan®probe ID)	
Pluripotency Markers (qPCR)	NANOG	109 bp	Thermo Fisher Cat# Hs02387400_g1 (TaqMan®probe ID)	
	POU5F1	77 bp	Thermo Fisher Cat# Hs04260367_gH (TaqMan®probe ID)	
	SOX2	91 bp	Thermo Fisher Cat# Hs01053049_s1 (TaqMan®probe ID)	
House-Keeping Genes (qPCR)	RPL13A	113 bp	Thermo Fisher Cat# Hs04194366_g1 (TaqMan®probe ID)	
	ACTB	171 bp	Thermo Fisher Cat# Hs99999903_m1 (TaqMan®probe ID)	

Table 2 (continued)

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Mycoplasma	ARN 16S	464 bp	GGCGAATGGGTGAGTAACACG/CGGATAACGCTGCGACCTATG	

4.5. Gene expression analysis

RNA samples (at passages 15 to 17) were extracted using the NucleoSpin Tissue Purification Kit (MACHEREY-NAGEL) and reverse transcribed using the High-Capacity cDNA Reverse-Transcription Kit (Applied Biosystems™). Quantitative PCR were conducted in duplicate using the Universal PCR Master Mix (Applied Biosystems™) and Taqman™ probes with a QuantStudio5 real-time PCR system (Applied Biosystems) (Table 2) using the standard mode, consisting of a hold stage at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of a PCR stage at 95 °C for 15 sec then 60 °C for 1 min. Data were normalized using ACTB and RPL13A as housekeeping genes. For pluripotent markers, gene expression was compared with a previously validated ITXi001-A hiPS cell line (Bray et al., 2022).

4.6. Flow cytometry

ITXi0012-A cells at passage 16 were dissociated using the Gentle Cell Dissociation Reagent (STEMCELL™ Technologies) and washed three times using FACS Buffer (PBS containing 0,1% BSA). Cells were incubated with PE-labeled antibodies (Table 2) for 1 h at 4 °C in the dark and further washed three times with FACS buffer. Analysis of the stained cells was performed using flow cytometry (BD FACSMelody™, BD). Unstained cells were used as negative controls and results were analyzed using FlowJo™ software (BD).

4.7. Immunofluorescent staining

ITXi0012-A cells at passage 16 were fixed with 4% paraformaldehyde (Sigma-Aldrich) for 30 min at room temperature (RT). After washing three times with PBS, cells were permeabilized with 0.5% Triton X-100 (Sigma-Aldrich) for 15 min and blocked with PBS BSA 3% for 30 min at RT then incubated with primary antibodies overnight at 4 °C. The day after, cells were washed twice with PBS and incubated for 1 h at room temperature with secondary antibodies (Table 2) and DAPI (Invitrogen™) for nuclear counterstaining. Images were captured using Eclipse Ti2 fluorescence microscope (Nikon) using Nikon Standard software.

4.8. Trilineage differentiation

ITXi0012-A cells at passage 17 were differentiated into three germ layers using the STEMdiff™ Trilineage Differentiation Kit (Miltenyi Biotec). RNA samples were extracted as previously described and the differentiation-markers analyzed using the Taqman™ probes listed in Table 2.

4.9. Cell line authentication test

Short tandem repeat (STR) analyses (16 STR tested) were performed on cell pellets from originally sampled urine progenitor cells (Ucell) and ITXi0012-A (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

Figure 1

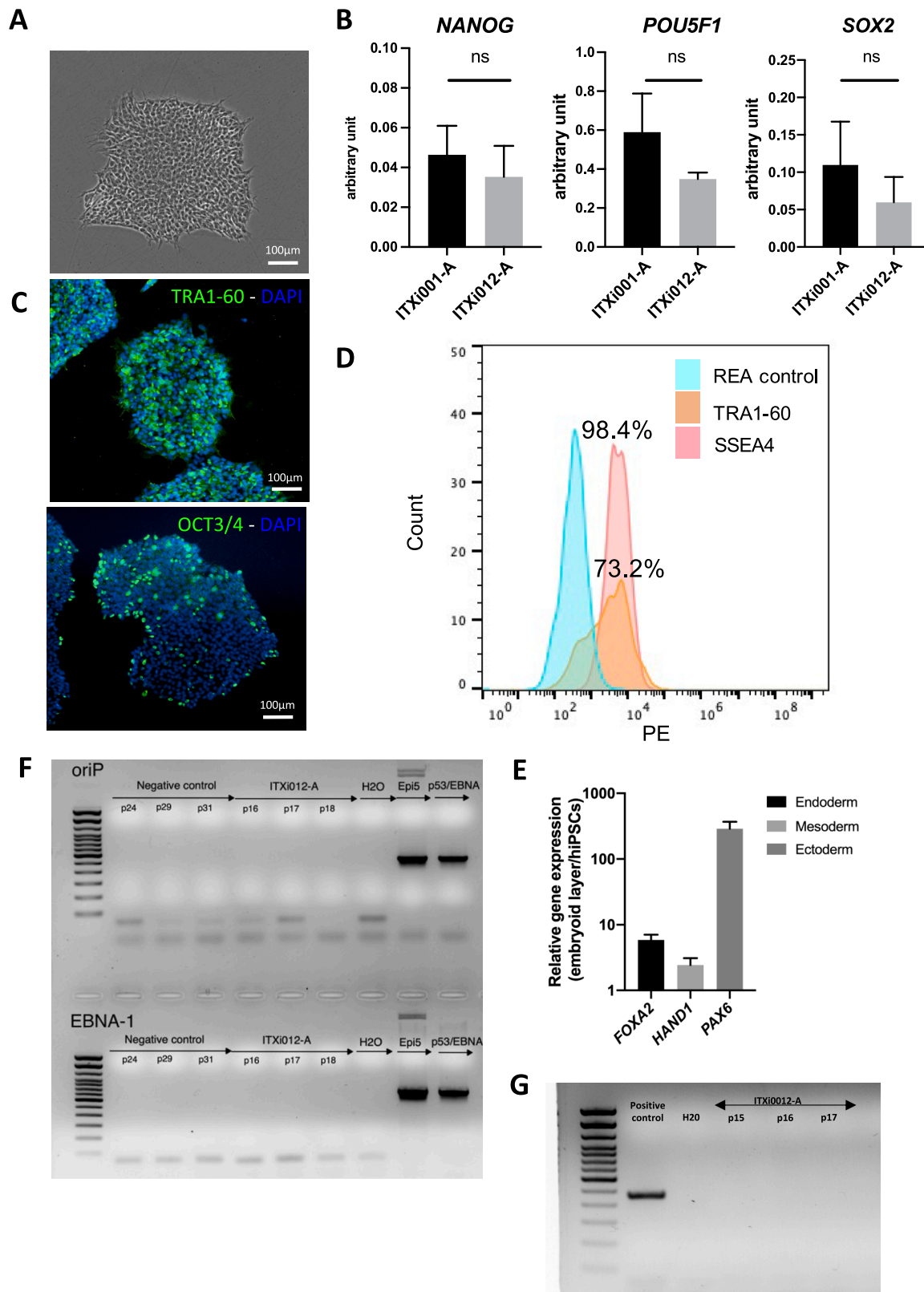


Fig. 1. Characterization of the ITXi012-A cell line.

the work reported in this paper.

Acknowledgments

This work was supported by the French national project CHOPIN (CHolesterol Personalized INnovation) funded by the Agence Nationale de la Recherche (ANR-16-RHUS-0007) and coordinated by the CHU of Nantes; the INSTINCTIVE research program funded by the Fondation pour la Recherche Médicale (FRM: EQU201903007846); the GENESIS and KRINGLE2 projects, funded by the Agence Nationale de la Recherche (ANR-21-CE14-0051 and ANR-20CE14-0009 respectively); the 3D Stem Serendipity Project founded by NExT Innovation Lab and coordinated by the CHU of Nantes and the foundation GENAVIE.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2023.103205>.

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