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Lab Resource: Genetically-Modified Single Cell Line

Generation of mutation-corrected induced pluripotent stem cell lines derived from adrenoleukodystrophy patient by using homology directed repair

Eul Sik Jung [a,1], Ji Hun Kim [b,1], Mi-Yoon Chang [c,d,1], Wonjun Hong [e], Zhejiu Quan [b], Seung Hyun Kim [b], Seungkwon You [e], Dae-Sung Kim [f], Jiho Jang [g], Sang-Hun Lee [c,d], Hyongbum Henry Kim [a], Hoon Chul Kang [b,*]

[a] Department of Pharmacology, Yonsei University College of Medicine, Seoul, Republic of Korea
[b] Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
[c] Department of Biochemistry and Molecular Biology, College of Medicine, Hanyang University, Seoul, Republic of Korea
[d] Hanyang Biomedical Research Institute, Hanyang University, Seoul, Republic of Korea
[e] Laboratory of Cell Function Regulation, Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea
[f] Department of Biotechnology, College of Life Science and Biotechnology, Korea University, Seoul, Republic of Korea
[g] Department of Physiology and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea

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ABSTRACT

X-linked adrenoleukodystrophy (ALD) caused by the *ABCD1* mutation, is the most common inherited peroxisomal disease. Previously, we generated an ALD patient-derived **SCHi001-A** iPSC model. In this study, we have performed the first genome editing of ALD patient-derived **SCHi001-A** iPSCs using homology-directed repair (HDR). The mutation site, c.1534G > A [GenBank: NM_000033.4], was corrected by introducing ssODN and the CRISPR/Cas9 system. The cell line exhibited normal iPSC plulipotency marker expression following genome editing. Mutation-corrected iPSCs from **SCHi001-A** iPSC line can be used in research into the pathophysiology of and therapeutics for ALD.

Resource Table

Unique stem cell line identifier	SCHi001-A-1
Alternative name(s) of stem cell line	AMN5-Corrected iPSC
Institution	Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Epilepsy Research Institute
Contact information of distributor	Hoon-Chul Kang, HIPO0207@yuhs.ac
Type of cell line	iPSC
Origin	Human
Additional origin info	Applicable for human ESC or iPSC Age: 34YR Sex: Male Ethnicity if known: Korean
Cell Source	Original cell type corrected: SCHi001-A iPSC
Method of reprogramming	CytoTune-iPS 2.0 Sendai Reprogramming vector
Associated disease	X-linked adrenoleukodystrophy (X-ALD)
Gene/locus	For human disease: ABCD1 mutation(c.1534G > A)
Alternative name(s) of stem cell line	AMN5-Corrected iPSC
Institution	Division of Pediatric Neurology, Department of Pediatrics, Severance Children's Hospital, Epilepsy Research Institute
Contact information of distributor	Hoon-Chul Kang, HIPO0207@yuhs.ac
Type of cell line	iPSC
Method of modification/site-specific nuclease used	Homology direct repair: CRISPR/Cas9
Site-specific nuclease (SSN) delivery method	Transfection
All genetic material introduced into the cells	CRISPR/Cas plasmid, HDR donor vector Sequencing of the targeted allele

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* Corresponding author.
E-mail address: HIPO0207@yuhs.ac (H. Chul Kang).
[1] These authors contributed equally to this work.

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(*continued*)

Analysis of the nuclease-targeted allele status	
Method of the off-target nuclease activity surveillance	Whole genome sequencing
Inducible/constitutive system details	N/A
Date archived/stock date	7 June 2018(passage No.: 42)
Ethical/GMO work approvals	Ethical committee: Yonsei University Health System, Severance Hospital, Institutional Review Board Approval number: 4-2016-0194

1. Resource utility

This SCHi001-A iPSC, derived from X-linked adrenoleukodystrophy (X-ALD) patient's fibroblast, is a mutation-corrected by genome editing technology with clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR/Cas9). SCHi001-A-1 iPSCs from ALD patient can be used in research into the pathophysiology of and therapeutics for ALD.

2. Resource details

X-linked adrenoleukodystrophy (ALD, OMIM:300100), a rare genetic disease, is manifested with inflammatory cerebral demyelination, progressive myeloneuropathy, and endocrine dysfunction such as an adrenal insufficiency or gonadal dysfunction (Bezman et al.,2001). Also, in adults, it develops into adrenal myelogenous neuropathy (AMN) whose main symptom is spinal cord symptoms. It is same as ALD, but it is called AMN when there is damage to the spine (Spurek et al., 2004). There is no curative option for this genetic disease. Previously, we reported the generation of an ALD patient fibroblast derived SCHi001-A iPSC model (Son et al., 2017).

In here, the CRISPR/Cas9 were used gene editing (Li et al., 2016) and sanger sequencing for genomic DNA confirmed the corrected c.1534G > A mutation to c.1534A > G (Fig. 1 F). In here we performed the mutation correction for ALD patient derived iPSC with the mutation, c.1534G > A. For the Off-target analysis, we performed WES to investigate the overall off-target effects of CRISPR/Cas9 introduction. The number of single nucleotide polymorphisms (SNPs) and variants from YUSEVi005-corrected A iPSC cell lines were similar to SCHi001-A iPSC (Supplementary Table 1). We analyzed the on-target reads of SCHi001-A iPSC iPSC in comparison with untreated SCHi001-A iPSC (c.1534G > A). Eight significant variants were captured for two for SCHi001-A, and none for DSC3 (Supplementary Table 2). These variants were not in crucial genes (Supplementary Table 2). The pluripotency of the SCHi001-A-1 iPS line was verified by immunocytochemistry assays, that had intense pluripotency markers expression, including OCT3/4, SOX2, TRA-1-81, SSEA4, TRA-1-60, and NANOG (Fig. 1 B). The karyotype was normal without abnormalities in the number or structure of chromosomes (Fig. 1 G). The SCHi001-A-1 iPSC line was negative for *Mycoplasma* contamination (Fig. 1 H). STR analysis showed that parental SCHi001-A and the corrected YUSEVi005-corrected A shared alleles with 100% match (Supplementary Table 2).

3. Materials and methods

3.1. Ethics statement

All experiments were conducted under the supervision of the Human Research Protection Center, Yonsei University College of Medicine, and followed the guidelines of the Institutional Review Board (Approval No. 4-2016-0194). The patient's skin sample was donated voluntarily.

3.2. Establishment of SCHi001-A iPSC lines from human fibroblasts

Recently, we reported establishmented SCHi001-A iPSC lines from human fibroblasts (Table 1) (Son et al., 2017).

3.3. Genome editing using HDR in patient-derived SCHi001-A iPSCs

We used feeder-free conditions for the genome editing experiment. Essential 8 medium (Life Technologies, CA, US) was used for feeder-free culture. Transfections were performed using the NEPA21 electroporator (Nepagene, Japan) and cells were then plated onto a Matrigel-coated 60-mm dish with Essential 8 medium. After allowing the cells to settle for 3 h, single isolated cells were marked with 3-mm circle markers on the bottom of the plate. Only colonies grown within the marked circles were used for further analysis. RFP + cells appeared within 24 h after electroporation. 12 h after transfection, cells were subjected to puromycin selection (1 μg/ml) for 3 h and the medium was subsequently changed to remove puromycin. Colonies after puromycin selection were maintained for 10 days. Then, perform serial passaging procedure to get homology mutation-corrected iPSCs. (Fig. 1 E)

3.4. Off-target analysis

At first, we performed in silico off-target analysis using Cas-OFFinder (http://www.rgenome.net/cas-offinder/) (Bae et al., 2014). Off-target analysis permitted two base pair mismatches and one DNA bulge in Cas-OFFinder. All possible on-target and off-target mutations were also analyzed by whole exome sequencing (WES). The exons were captured using the Agilent Sureselect kit, version V6, which covers the exomes of more than 20,000 genes. These were sequenced on an Illumina Novaseq 6000 sequencing system and the reads were mapped to the human reference genome GRCh37. We called variants using the Haplotype Caller algorithm from the Genome Analysis Toolkit (GATK) (3.8-0) (http://www.broadinstitute.org/gatk/).

3.5. Sequencing analysis of the corrected ABCD1 alleles and Karyotyping

The genomic DNA were carried out from fibroblast and iPSC. Target site in genomic DNA were amplified using an AccuPower PCR PreMix kit (Bioneer, Daejeon, Korea, Cat. No.: K2016) according to the manufacturer's instructions respectively. They PCR product for AMN5 (742 bp) sequences confermed by CosmoGenetech Inc. Korea. Primers used were as follows: AMN5 (742 bp; NG_009022.2: 19,921 to 20662) primers, forward: 5′-CTGTGGCAGAATAGGCCCTT-3′; reverse: 5′-CTCCCCCAAGATACTCTGCG-3′. PCR were performed for 30 cycles under the following conditions: denaturation at 94℃ for 30 s, annealing at 58℃ for 30 s, and extension at 72℃ for 50 s. Karyotyping was performed by GTG banding by standard G-banding technique (GenDix, Inc. Seoul, Korea).

3.6. Mycoplasma contamination detection

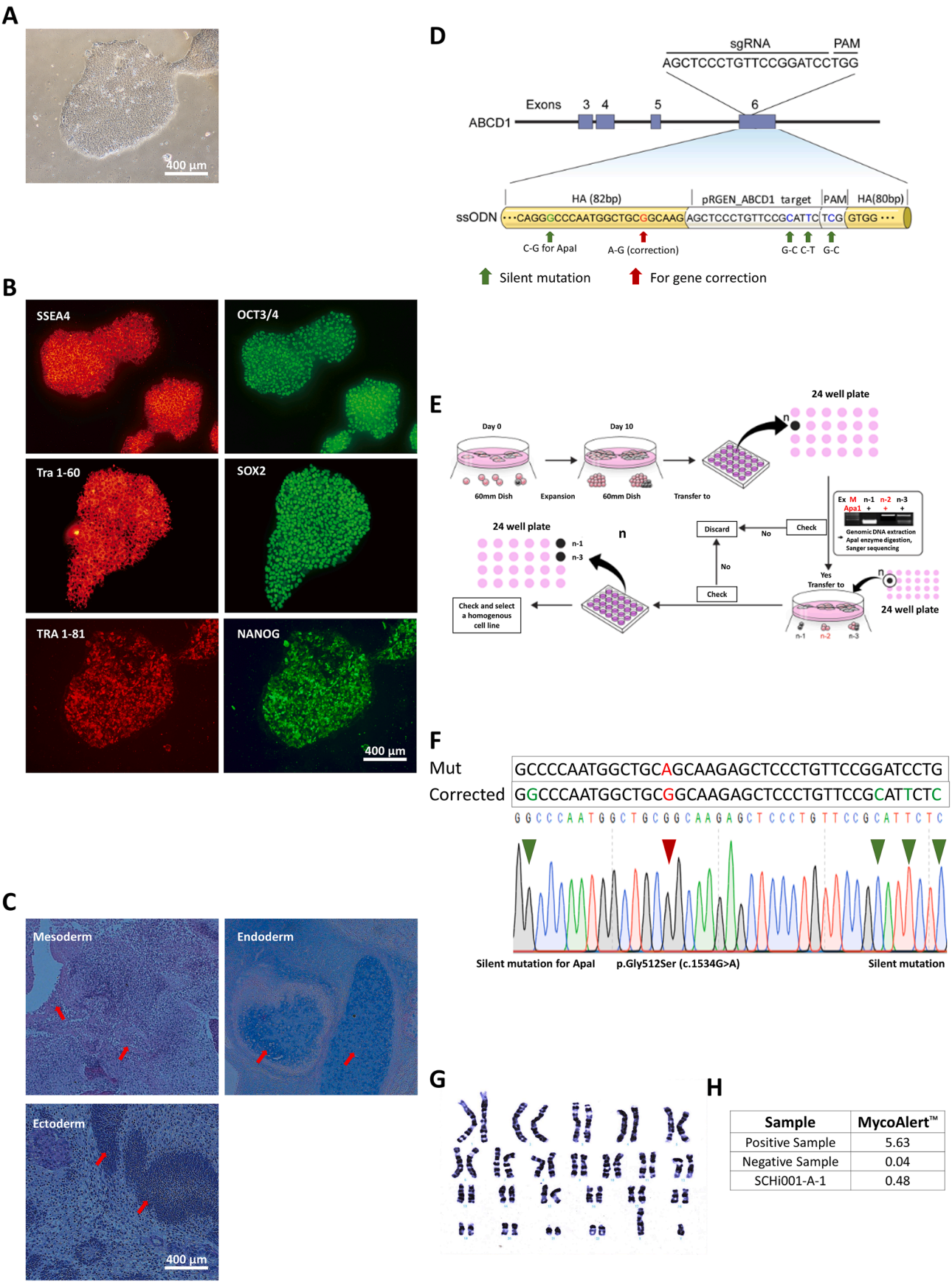
The absence of mycoplasma contamination was confirmed using MycoAlert™ PLUS Mycoplasma Detection kit (Lonza).

3.7. STR analysis

The **SCHi001-A** and **SCHi001-A-1** iPSC were authenticated using STR analysis by BIONICS (see Table 2).

4. Reagents details

Sequences of single-stranded oligonucleotide donor templates (ssODN) used in HDR: TCTCTGGCGTCAGCGGCTGTTGCCCCTGCAGGTGGAGGAAGGCATGCATCTGCTCATCA-CAGG**G**CCCAATGGCTGCGGCAAGAGCTCCCTGTTCCGCAT



Sample	MycoAlert™
Positive Sample	5.63
Negative Sample	0.04
SCHi001-A-1	0.48

Fig. 1. Establishement of gene corrected iPSC derived from X-linked adrenoleukodystrophy (ALD) patient.

Table 1
generated iPSC lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
SCHi001-A	AMN 5	Male	34	Korean	p.Gly512Ser (c.1534G > A)	X-ALD
SCHi001-A-1	AMN 5 corrected	Male	34	Korean	p.Gly512Gly (c.1534A > G)	corrected

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line: normal	Fig. 1 panel A
Phenotype	Immunocytochemistry	Assess staining/expression of pluripotency markers: OCT4, NANOG, TRA-1-81	Fig. 1 panel B
Differentiation potential	In vivo differentiation	Confirm Mesoderm, Edoderm, Ectoderm	Fig. 1 panel C
corrected analysis (IF APPLICABLE)	Sequencing	homozygote	Fig. 1 panel D
Homology SCHi001-A iPSC line selection	Serial passaging	homozygout	Fig. 1 panel E F
Genotype	Karyotype (G-banding) and resolution	46 XY, Resolution 450 (YUSEVi005-corrected A)	Fig. 1 panel G
Off target analysis	Wholegenomexon sequencing	2 mismatch	Supplementary Table 3
	STR analysis	20 locus tested. 100% match	Submitted in archive with journal
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Fig. 1 panel H
Differentiation potential	In vivo differentiation	Teratoma formation and IHC	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	

*TC*TCGGTGGGCTCTGGCCCACGTACGGTGGTGTGCTCTA-CAAGCCCCCACCCCAGCGCATGTTCTACATCCCGCAGAGGTAAGGA

Double Underline: sgRNA sequence
bold: silent mutation for ApaI restriction enzyme
italic: to prevent re-cleavage by sgRNA-Cas9 complex
Underline: PAM site

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat# and RRID
Pluripotency markers	Anti-Oct-4 Antibody	1 : 500	Millipore Cat# AB3209, RRID: AB_2167706
Pluripotency markers	Anti-Stage-Specific Embryonic Antigen-4 antibody	1 : 500	Millipore Cat# MAB4304, RRID:AB_177629
Pluripotency markers	Anti-Sox2 Antibody	1 : 500	Millipore Cat# AB5603, RRID:AB_2286686
Pluripotency markers	Anti-TRA-1–60 Antibody, clone TRA-1–60	1 : 500	Millipore Cat# MAB4360, RRID:AB_2119183
Pluripotency markers	Anti-TRA-1–81 Antibody	1 : 500	Millipore Cat# MAB4381, RRID:AB_177638
Pluripotency markers	Anti-Nanog Antibody	1 : 500	Millipore Cat# AB9220, RRID:AB_11213156
Secondary	Alexa Fluor 488-conjugated Donkey Anti-Mouse IgM	1 : 1000	Thermo Fisher Scientific Cat# A32766, RRID: AB_2762823
Secondary	Alexa Fluor 568-conjugated Donkey Anti-Rbbit IgM	1 : 1000	Thermo Fisher Scientific Cat# A10042, RRID: AB_2534017
Primers			
	Target	Forward/Reverse primer (5′-3′)	
Targeted mutation analysis/sequencing(**SCHi001-A**)	ABCD1(742 bp; NG_009022.2: 19,921 to 20662)	CTGTGGCAGAATAGGCCCTT/CTCCCCCAAGATACTCTGCG	
Targeted corrected analysis/sequencing(**SCHi001-A-1**)		CTGTGGCAGAATAGGCCCTT/CTCCCCCAAGATACTCTGCG	
sgRNA (pRGEN_*ABCD1)*	ABCD1 (Exon , bp)	AGCTCCCTGTTCCGGATCC	

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.2022.102664.

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