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Lab Resource: Multiple Cell Lines

Generation of a set of isogenic iPSC lines carrying all APOE genetic variants (Ɛ2/Ɛ3/Ɛ4) and knock-out for the study of APOE biology in health and disease

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A B S T R A C T

APOE genotype is the strongest genetic risk factor for Alzheimer's Disease (AD). The low degree of homology between mouse and human APOE is a concerning issue in preclinical models currently used to study the role of this gene in AD pathophysiology. A key objective of ADAPTED (Alzheimer's Disease Apolipoprotein Pathology for Treatment Elucidation and Development) project was to generate *in vitro* models that better recapitulate human APOE biology. We describe a new set of induced pluripotent stem cells (iPSC) lines carrying common APOE variants (Ɛ2, Ɛ3, and Ɛ3/Ɛ4) and a knock-out isogenic to the parental APOE Ɛ4/Ɛ4 line (UKBi011-A).

Resource Table

Unique stem cell lines identifier	1. EBiSC reference UKBi011-A-1, Biosample ID SAMEA104243174 2. EBiSC reference UKBi011-A-2, Biosample ID SAMEA104243175 3. EBiSC reference UKBi011-A-3, Biosample ID SAMEA104243176 4. EBiSC reference UKBi011-A-4, Biosample ID SAMEA104243177
Alternative names of stem cell lines	1. EBiSC reference UKBi011-A-1: APOE-KO 2. EBiSC reference UKBi011-A-2: APOE-2/2 3. EBiSC reference UKBi011-A-3: APOE-3/3 4. EBiSC reference UKBi011-A-4: APOE-3/4
Institution	Bioneer A/S
Contact information of distributor	Benjamin Schmid, bsc@bioneer.dk
Type of cell lines	iPSC
Origin	Human
Cell Source	iPSC
Clonality	Clonal
Method of reprogramming	CytoTune-iPS 2.0 Sendai Reprogramming Kit
Multiline rationale	Isogenic clones
Gene modification	YES
Type of modification	Single base change
Associated disease	Alzheimer's Disease
Gene/locus	APOE; 19q13.32; rs7412; rs429358
Method of modification	CRISPR
Name of transgene or resistance	CRISPR/CAS9
Inducible/constitutive system	N/A
Date archived/stock date	December 2019
Cell line repository/bank	https://www.ebisc.org/
Ethical approval	https://www.ebisc.org/

(*continued on next column*)

Resource Table (*continued*)

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1. Resource utility

We have previously generated a set of isogenic iPSC lines for the study of human APOE biology (Schmid et al., 2019). We describe here a new set of iPSC lines derived from an Alzheimer's disease patient with genotype APOE 4/4 Resource Table. These lines are useful for replicating previous and discovering new aspects of APOE biology (Table 1).

2. Resource details

The starting material used to generate the set of APOE-gene edited lines was the iPSC line UKBi011-A, generated from an 80 years old AD patient with an APOE 4/4 genotype, which has already been described (Peitz et al., 2018). The lines with the genotypes APOE 3/4 and APOE 3/3 as well as the KO line were generated from the parental line with an APOE 4/4 genotype. The line with the APOE 2/2 genotype was then generated from the APOE 3/3 line (Tables 2 and 3).

After gene-editing, the identity of all clones was confirmed by STR analysis (submitted to Journal, data not published). All clones showed a normal karyotype by G-banding analysis, and no aberrations were found by SNP analysis (Fig. 1A). Pluripotency was assessed by staining with SOX2 and OCT4 at the pre-differentiated stage (Fig. 1C). We additionally quantified pluripotency of each cell line by performing qRT-PCR for *POUF51, NANOG* and *SOX2* in pre-differentiated cells compared to reference lines (Fig. 1F). On each one of the four clones, we also assessed differentiation potential by using a combination of immunocytochemical markers defining each germ layer: KRT18 & SOX17 for endoderm, TBXT (Brachyury) & SMA (smooth Muscle Actin) for mesoderm and PAX6 & NES for ectodermal lineage (Fig. 1B). The genotype of each clone was confirmed by sanger sequencing (Fig. 1D). The absence of APOE protein for the KO clone was demonstrated by western blot (Fig. 1D). As a further validation step, we confirmed all genotypes by TaqMan technology using specific assays detecting each APOE allele (Fig. 1E).

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Normal*	*not shown but available with author*
Phenotype	Immunocytochemistry	Assess staining/ expression of pluripotency markers: Oct4 and Sox2	Fig. 1 panel B
	Quantitative RT-PCR analysis	Assess % of transcripts for pluripotent markers e.g. Oct3/4, NANOG and SOX2	Fig. 1 panel F
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 400–500	Fig. 1 panel A
Identity	STR analysis	Performed	Supplementary file
		16 sites tested, all matched	e.g. submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing and TaqMan	All genotypes confirmed	e.g. Fig. 1 panel E
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR: Negative	Fig. 1/ Supplementary
Differentiation potential	Directed differentiation	Proof of three germlayer formation.	e.g Fig. 1 panel B and C

3. Materials and methods

3.1. Gene-editing

For each genotype, a specific combination of CRISPR and a homologous construct was used:

1) For the lines UKBi011-A-4 and UKBi011-A-3 (APOE 3/4 and APOE 3/3), we nucleofected the parental line UKBi011-A with the CRISPR "CRISPR2_recC" targeting the C at rs429358 and the homologous construct "ssODN rs429358-T" containing a T at rs429358.
2) For the line UKBi011-A-2 (APOE 2/2), we nucleofected the line UBi011-A-3 (APOE 3/3) with the CRISPR "CRISPR3_recC" targeting the C at rs7412 and the homologous construct "ssODN rs7412-T" containing a T at rs429358.
3) For the line UKBi011-A-1 (APOE KO), we nucleofected the parental line UKBi011-A with CRISPR1 targeting Exon2 of the APOE gene and the homologous construct "ssODN_ApoE-KO_BclI_Cr1", which contains 8 additional bases consisting of a stop codon, a Bcl1 recognition site and 2 additional random bases.

The RNA strands for the CRISPRs were ordered at IDT as crRNA and tracrRNA. The RNA was mixed in a ratio of 1:1 (2 μL each of 200 μM concentration) and heated up to 95 °C for 5 min. Then, the RNA was brought back to room temperature to form the assembled RNA constructs (sgRNAs). 2 μL of the sgRNA (100 μM) were mixed with 2 μL of Cas9 protein (IDT, Cat # 1081067) to form the active CRISPR/Cas9 complex.

iPSCs were cultured in 6 well plates coated with Matrigel (Corning Bioscience) in E8 medium and detached using Accutase (Gibco). When they reached a density of 70–90%, a total of 1.5×10^6 cells were co-nucleofected with 4 μL of the CRISPR/Cas9 complex and 2 μL of the ssODN (100 μM). For the nucleofection, the P3 Primary Cell Kit (Lonza) was used and run with the program CA167 following manufacturer's instructions. iPSCs were subsequently transferred back to a Matrigel-coated 100 mm dish in E8 medium supplemented with 1:200 diluted Revita cell supplement (Gibco). After 6–7 days, iPS colonies were picked in a 96 well plate and expanded for genotyping.

3.2. Screening

DNA for genotyping was extracted using the QIAGEN protease (Cat# 19155) following the manufacturer's instructions in a volume of 10 μL. 100 μL of water was added to the extracted DNA. Genotyping was done

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
UKBi011-A-1	APOE-KO	Male	80	Caucasian	APOE	Alzheimer's Disease
UKBi011-A-2	APOE-2/2	Male	80	Caucasian	APOE	Alzheimer's Disease
UKBi011-A-3	APOE-3/3	Male	80	Caucasian	APOE	Alzheimer's Disease
UKBi011-A-4	APOE-3/4	Male	80	Caucasian	APOE	Alzheimer's Disease

Table 3
Reagents details *RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in table as shown in examples.*

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Anti-Oct-4A (C30A3) Antibody	Rabbit anti-OCT4	1:40	Cell Signaling Technology Cat#2840
Anti-Sox2 (D6D9) antibody	Rabbit anti-SOX2	1:200	Cell Signaling Technology Cat#3579S
Anti-Cytokeratin-18 antibody	Rabbit anti-KRT8	1:300	Abcam Cat# ab52948
Anti-SOX17 antibody [3B10]	Mouse anti-SOX17	1:50	Abcam Cat# ab84990
Human/Mouse Brachyury Antibody	Goat anti-Brachury	10 µg/ml	R&D Systems Cat# AF2085
Anti-Human Smooth Muscle Actin Antibody	Mouse anti-SMA	1:50	DAKO, Agilent Cat# M085129-2
anti-Pax-6 Antibody	Rabbit anti-PAX6	1:150	Biolegend Cat# 901301
Anti-Nestin Antibody	Mouse anti-Nestin	1:50	Novus Biologics Cat# NB300-266
Secondary antibodies	Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	*1:1000*	Thermofischer Cat# A-21206
	Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555		Thermofischer Cat# A-31572
	Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555		Thermofischer Cat# A-31570
	Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488		Thermofischer Cat# A-11055
APOE antibody	Apolipoprotein E/APOE Antibody	1:1000	NOVUS Biologicals; NB110-60531 RRID:AB_920623
sgRNAs, ssODNs and primers	**Target**	**Forward/Reverse primer (5′-3′)**	
CRISPR1	Exon2	GGTTCTGTGGGCTGCGTTGCTGG	
CRISPR2_recT	rs429358	GCGGACATGGAGGACGTGTGCGG	
CRISPR2_recC	rs429358	GCGGACATGGAGGACGTGCGCGG	
CRISPR3_recC	rs7412	ACACTGCCAGGCGCTTCTGCAGG	
ssODN rs429358-T	rs429358	AGGAGCTGCAGGCGGCGCAGGCCCGG-CTGGGCGCGGACATGGAGGACGTGTG-CGGCCGCCTGGTGCAGTACCGCGGCG-AGGTGCAGGCCATGCTCGGCCAG	
ssODN rs429358-C	rs429358	AGGAGCTGCAGGCGGCGCAGGCCCGG-CTGGGCGCGGACATGGAGGACGTGCG-CGGCCGCCTGGTGCAGTACCGCGGCG-AGGTGCAGGCCATGCTCGGCCAG	
ssODN rs7412-T	rs7412	TGCGCAAGCTGCGTAAGCGGCTCCTCC-GCGATGCCGATGACCTGCAGAAGTGCC-TGGCAGTGTACCAGGCCGGGGCCCGCG-AGGGCGCCGAGCGCGGCCTC	

Table 3 (*continued*)

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
ssODN_ApoE-KO_BclI_Cr1	Exon2	CCCAGACTGGCCAATCACAGGCAGGAA-GATGAAGGTTCTGTGGGCTGCGTGATCA-CTTGCTGGTCACATTCCTGGCAGGTATGG-GGGCGGGGCTTGCTCGGTTCCCC	
APOE HhaI	rs 429358; rs7412	GCACGGCTGTCCAAGGAG/GCCCCGGCCTGGTACAC	
APOE HhaI Seq	rs 429358; rs7412	TGTCCAAGGAGCTGCAGG	
SURV APOE KO	APOE exon2	GAACACGGCGCTTAACTGTG/CAGAGAGCGTCAAATCGCTGT	
SURV APOE KO Seq	APOE exon2	GCGGCTTGGTAAATGTGCTG	
U6-FW	pSpCas9n(BB)-2A-Puro plasmid	GAGGGCCTATTTCCCATGATTCC	

by PCR, RFLP and sequencing analysis. The PCR was carried out using AmpliTaq Gold Polymerase (Thermo Fisher) according to the manufacturer's instructions at an annealing temperature of 60° C and an elongation time of 30 s. For the APOE 2/2, 3/3 and 3/4 genotypes, the primers "APOE HhaI" were used. PCR products were digested with the enzyme HhaI (NEB) and run on a 4% agarose gel for 45 min at 90 V and patterns were identified according to previous publications (Hixson and Vernier, 1990). Positive candidates were then sequenced using the sequencing primer "APOE HhaI Seq". For the APOE KO clone, the primers "SURV APOE KO Seq" were used. PCR products were digested with BclI (NEB) and run on a 2% agarose gel for 35 min at 90 V. Clones, which were positive in the RFLP analysis were then sequenced using the primer "SURV APOE KO Seq".

Single cells were produced from all positive clones. The cells were incubated with Accutase (Gibco) for 4–5 min. Cells were triturated and pressed through a cell strainer (35 µm diameter, Stemcell technologies). The cells were counted, and 10,000 cells were plated on a 100 mm plate with 1:200 Revita cell supplement (Gibco). After one week, isolated colonies with a round shape were picked, re-screened as described above and monoclonal populations were identified and banked.

3.3. TaqMan APOE genotyping

APOE genotype was confirmed by using TaqMan technology, assay IDs 3084793_20 (SNP rs429358) and 904973_10 (SNP rs7412).

3.4. Pluripotency test

Pluripotency was assessed by immunocytochemical detection of pluripotency markers: Sox2 & Oct4. Differentiation of all lines into all three germ layers was assessed using three germline markers: Cytok18 & Sox17 for endoderm, Brachyury & Smooth Muscle Actin for mesoderm and Pax6 & Nestin for ectoderm lineage, using the STEMdiff™ Trilineage Differentiation Kit (StemCell Technologies) according to the manufacturer's instructions.

3.5. Immunofluorescence

Cells were fixed with 4% paraformaldehyde at RT for 15 min, washed three-times with PBS after which they were blocked with 10% FBS in PBS with 0.1% TritonX-100 for 1 h at RT. Primary antibodies were incubated overnight at 4 °C. After washing three times with PBS, the secondary antibody was incubated for 1 h at RT. Images were acquired using the Operetta CLS™ High-Content Analysis System and analysed using the associated Harmony Software. The following antibodies were used: Anti-Cytokeratin 18 (KRT18) antibody ab52948 (Abcam), Anti-SOX17 antibody [3B10] ab84990 (Abcam), Human/Mouse Brachyury Antibody AF2085 (R&D Systems), Anti-Human Smooth Muscle Actin Antibody M085129-2 (DAKO, Agilent), anti-PAX6 Antibody 901301

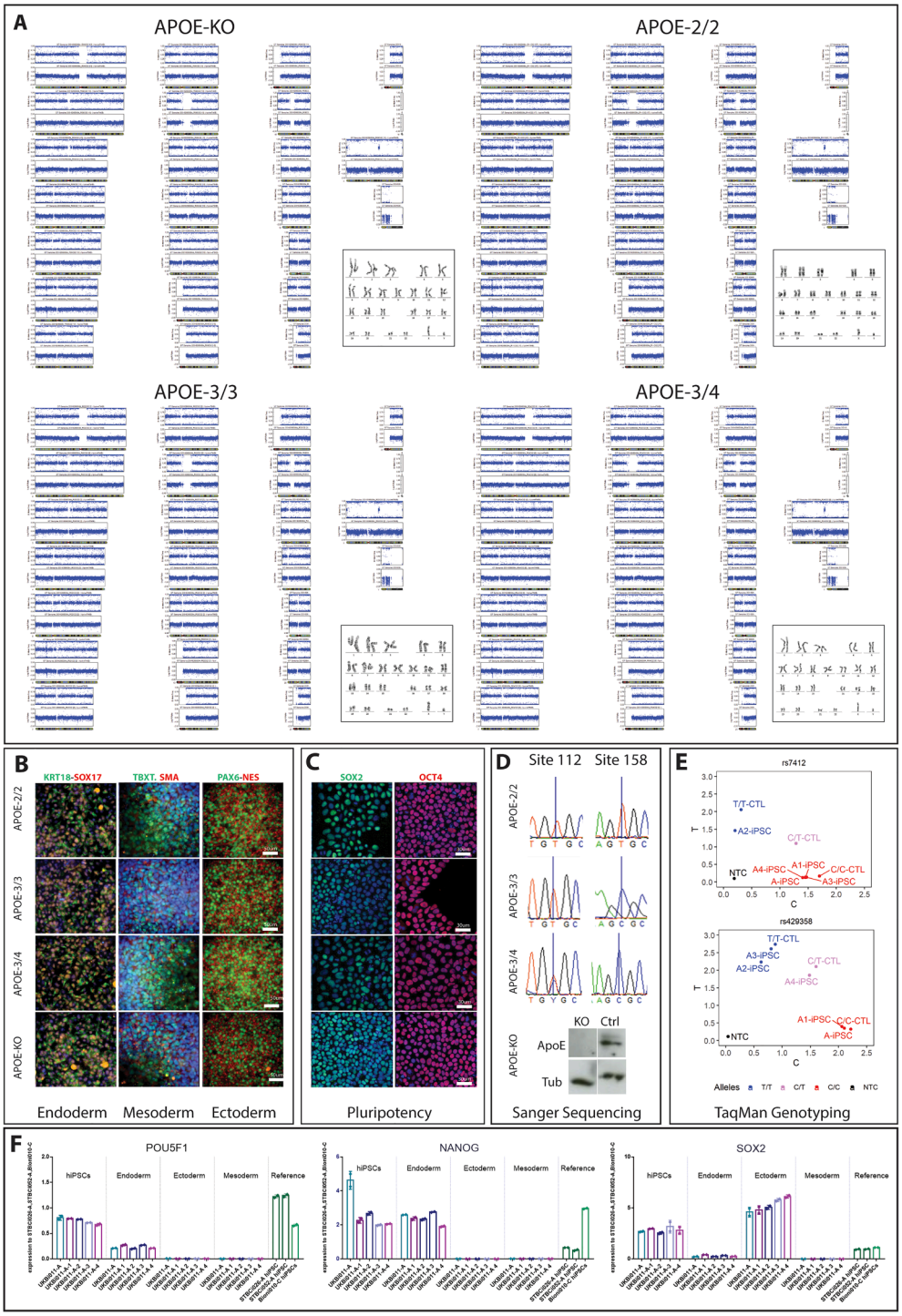

Fig. 1. Characterization and quality controls of APOE-edited lines UKBi011-A1, UKBi011-A2, UKBi011-A3 and UKBi011-A4. A, karyiotyping and SNP analysis; B, trilineage differentiation potential; C, pluripotency; D, sanger sequencing and western blot; E, TaqMan genotyping; and F, Quantitative RT-PCR analysis of pluripotency markers.

(Biolegend), Anti-Nestin Antibody (10C2) NB300-266 (Novus Biologics), Anti-SOX2 (D6D9) antibody 3579S (Cell Signaling Technology), Anti-OCT4A (C30A3) Antibody 2840, Cell Signaling Technology).

3.6. TaqMan pluripotency

In addition to the immunocytochemical staining, pluripotency as well as differentiation into all three germ layers was assessed by RT-qPCR for the expression of the following genes: *POU5F1, NANOG, SOX2, KLF4, NESTIN, PAX6, BRACHURY (TBRT), SMOOTH-MUSCLE-ACTIN (SMA), SOX17 and CYTOKERATIN-18 (KRT18)*. Displayed is fold-change relative to the previously characterized cell lines: STBCi026-A, STBCi052-A, Bioni010-C (all available at EBiSC repository).

RNA was collected and purified using RNeasy Plus Mini Kit (Qiagen Cat. # 74134).

RNA was converted into cDNA using SuperScript IV VILO Master-Mix (ThermoFisher Scientific Cat. # 11766500) and the following TaqMan

Probes were used:

1. ACTB; Hs99999903_m1
2. RPL13; Hs00744303_s1
3. NANOG; Hs02387400_g1
4. SOX2; Hs01053049_s1
5. SOX17; Hs00751752_s1
6. POU5F1; Hs04260367_gH
7. NES; Hs04187831_g1
8. PAX6; Hs01088114_m1
9. T; Hs00610080_m1
10. ACTA2; Hs00426835_g1
11. KRT18; Hs02827483_g1
12. REXO1; Hs00381890_m1
13. KLF4; Hs00358836_m1

ß-Actin (ACTB); Hs99999903_m1
RPL13; Hs00744303_s1

3.7. STR analysis

STR analysis was performed by Eurofins Medigenomix Forensik GmbH by using a PowerPlex 21 PCR kit (Promega).

3.8. Mycoplasma detection

Genomic DNA was extracted and analysed with the qPCR kit Venor GeM qEP (Minerva Biolabs).

3.9. SNP analysis

SNP genotyping was performed at the Institute of Human Genetics at the University of Bonn using an Infinium PsychArray-24 v1.1 BeadChip (Illumina). Data was analyzed using GenomeStudio (Illumina).

Acknowledgments

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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.2021.102180.

References

Schmid, B., Prehn, K.R., Nimsanor, N., Garcia, B.I.A., Poulsen, U., Jørring, I., Rasmussen, M.A., Clausen, C., Mau-Holzmann, U.A., Ramakrishna, S., Muddashetty, R., Steeg, R., Bruce, K., Mackintosh, P., Ebneth, A., Holst, B., Cabrera-Socorro, A., 2019. Generation of a set of isogenic, gene-edited iPSC lines homozygous for all main APOE variants and an APOE knock-out line. Stem Cell Res. 34, 101349. https://doi.org/10.1016/j.scr.2018.11.010.

Peitz, M., et al., 2018. Blood-derived integration-free iPS cell line UKBi011-A from a diagnosed male Alzheimer's disease patient with APOE varepsilon4/varepsilon4 genotype. Stem Cell Res. 29, 250–253. https://doi.org/10.1016/j.scr.2018.04.011.

Hixson, J.E., Vernier, D.T., 1990. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J. Lipid Res. 31 (3), 545–548. https://doi.org/10.1016/S0022-2275(20)43176-1.