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Lab resource: Stem cell line

Generation of 3 human induced pluripotent stem cell lines LUMCi005-A, B and C from a Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type patient

Elena Daoutsali[a,*], Ronald A.M. Buijsen[a], Simone van de Pas[b], Anke 't Jong[b], Harald Mikkers[b], Tom Brands[c,d], Bert Eussen[c], Annelies de Klein[c], Linda M. van der Graaf[a], Barry A. Pepers[a], Christian Freund[b], Gisela M. Terwindt[e], Valeria V. Orlova[f,1], Willeke M.C. van Roon-Mom[a,1]

[a] Department of Human Genetics, LUMC, Leiden, The Netherlands
[b] LUMC hiPSC Core Facility, Department of Anatomy and Embryology, LUMC, Leiden, The Netherlands
[c] Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands
[d] Department of Ophthalmology, Erasmus MC, Rotterdam, The Netherlands
[e] Department of Neurology, LUMC, Leiden, The Netherlands
[f] Department of Anatomy and Embryology, LUMC, Leiden, The Netherlands

A B S T R A C T

Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type (HCHWA-D) is an autosomal dominant hereditary disease caused by a point mutation in exon 17 of the *APP* gene. We generated human induced pluripotent stem cells (hiPSCs) from a symptomatic HCHWA-D patient by using non-integrating Sendai virus (SeV). The newly generated hiPSCs express all pluripotency markers, have a normal karyotype, carry the Dutch mutation, can differentiate in the three germ layers *in vitro* and are SeV free.

Resource table

Unique stem cell lines identifier	LUMCi005-A LUMCi005-B LUMCi005-C
Alternative names of stem cell lines	LUMCi005-A: LUMC0074iHCHWAD01 and Lu074i#1 LUMCi005-B: LUMC0074iHCHWAD03 and Lu074i#3 LUMCi005-C: LUMC0074iHCHWAD07 and Lu074i#7
Institution	Leiden University Medical Center (LUMC), Leiden, The Netherlands
Contact information of distributor	Dr. Willeke M.C van Roon-Mom, W.M.C.van_Roon@lumc.nl
Type of cell lines	hiPSCs
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrating Sendai virus
Multiline rationale	3 clones of one disease cell line
Gene modification	Yes
Type of modification	Hereditary
Associated disease	Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type (HCHWA-D)
Gene/locus	APP/21q21.3, Dutch mutation/ Chr21:25891856 G > C
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	21/09/2015
Cell line repository/bank	https://hpscreg.eu/cell-line/LUMCi005-A https://hpscreg.eu/cell-line/LUMCi005-B https://hpscreg.eu/cell-line/LUMCi005-C
Ethical approval	NL45478.058.13/P13.080, Medical Ethical Committee (MEC), Leiden University Medical Center (LUMC)

1. Resource utility

The generated hiPSCs are useful to study HCHWA-D disease mechanisms, as well as for drug discovery and disease modeling.

2. Resource details

Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type (HCHWA-D) is a hereditary autosomal dominant disease, clinically characterized by recurrent intracerebral hemorrhage (ICH) and

* Corresponding author.
E-mail address: e.daoutsali@lumc.nl (E. Daoutsali).
[1] Shared last authors.

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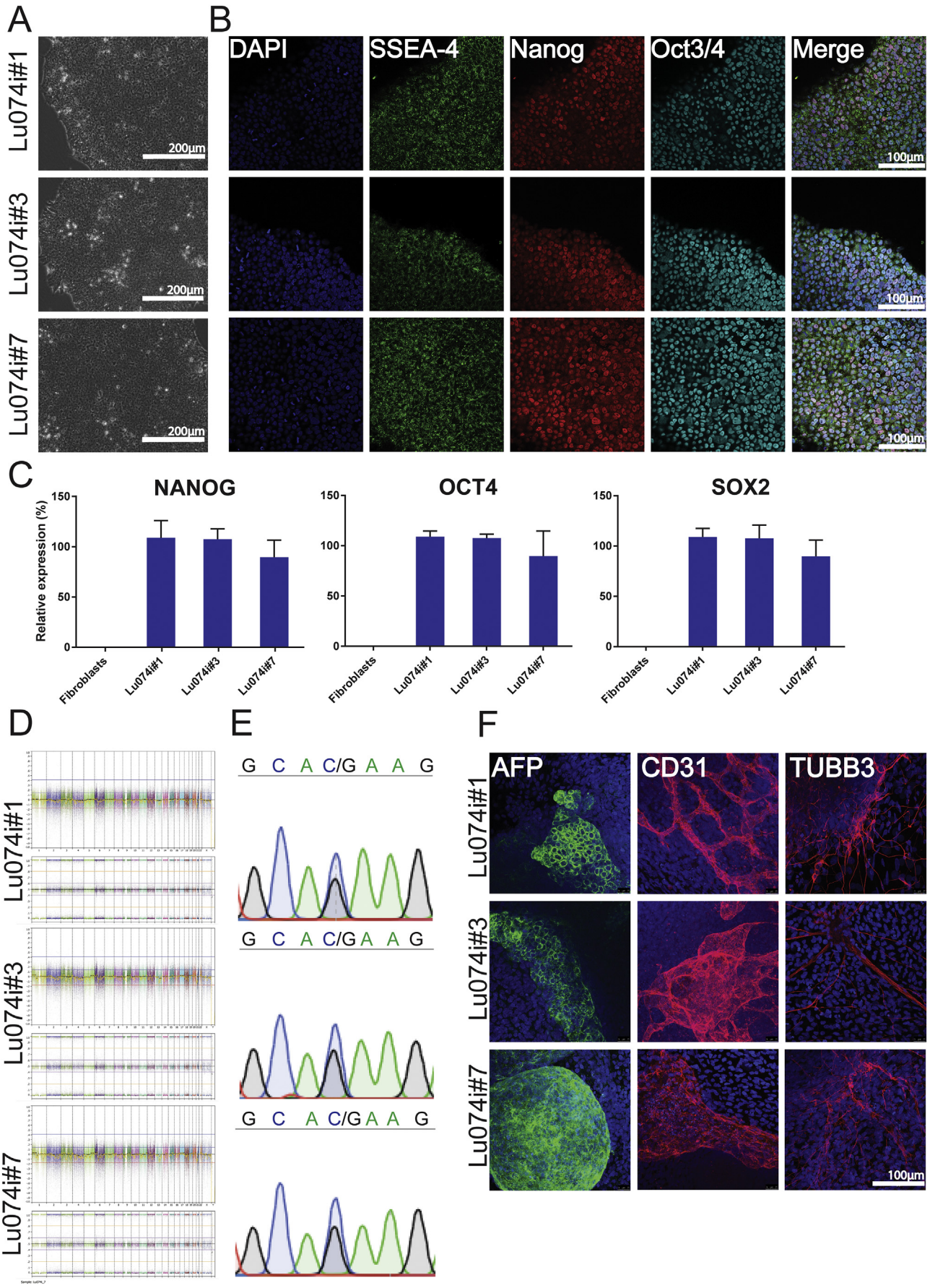
A
B
Lu074i#1
Lu074i#3
Lu074i#7
200μm
DAPI
SSEA-4
Nanog
Oct3/4
Merge
100μm
C
NANOG
OCT4
SOX2
Relative expression (%)
150
100
50
0
Fibroblasts
Lu074i#1
Lu074i#3
Lu074i#7
D
E
F
G C A C/G A A G
AFP
CD31
TUBB3

Fig. 1.

Table 1
Summary of lines.

hiPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
LUMCi005-A	Lu074i#1	Female	56	Caucasian	Chr21:25891856 G > C	HCHWA-D
LUMCi005-B	Lu074i#3	Female	56	Caucasian	Chr21:25891856 G > C	HCHWA-D
LUMCi005-C	Lu074i#7	Female	56	Caucasian	Chr21:25891856 G > C	HCHWA-D

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield microscopy	Normal morphology	Fig. 1 panel A
Phenotype	Qualitative analysis of immunofluorescent staining	Positive immunostaining of the pluripotency markers OCT4, NANOG and SSEA-4	Fig. 1 panel B
	Quantitative analysis by RT-qPCR	Expression of the pluripotency markers OCT4, NANOG and SOX2	Fig. 1 panel C
Genotype	GSAMD24 v1 Illumina Infinium SNP array 700 k	CNV report resolution ~50 kb: No major copy number variations or allelic changes for Lu074i#1, Lu074i#3 and Lu074i#7	Fig. 1 panel D
Identity	GSAMD24 v1 Illumina Infinium SNP array 700 k	Fibroblasts and hiPSCs have > 99,99% identical SNPs	Summarized data in Fig. S1. Raw data available with the authors.
Mutation analysis	Sanger Sequencing	All hiPSC clones have the heterozygous Chr21:25891856 G > C mutation	Fig. 1 panel E
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence: Negative	Fig. S1
Differentiation potential	Qualitative analysis of immunofluorescent staining	Positive immunostaining of the three germ layers markers AFP (endoderm), CD31 (mesoderm) and TUBB3 (ectoderm)	Fig. 1 panel F
Donor screening	N/A		
Genotype additional info	ApoE genotyping by melting curve analysis	All clones have ApoE genotype ApoE3/4	Data available with the authors

dementia with amyloid beta (Aβ) deposition in the cerebral leptomeningeal and cortical blood vessels. HCHWA-D mutation carriers usually get their first ICH at the age of 50 with multiple recurrences in the years thereafter. Because of the similarities in Aβ composition and clinical symptoms HCHWA-D is considered to be a model for sporadic cerebral amyloid angiopathy. HCHWA-D is caused by a G to C transversion in codon 693 of the APP gene that results in a Gln-to-Glu (NP_000475.1:p.Glu693Gln) amino acid substitution, that is located within the Aβ sequence of APP (E22Q) (Bornebroek et al., 1996; Kamp et al., 2014). Fibroblasts, derived from a patient skin biopsy were successfully reprogrammed into hiPSCs using a replication defective and persistent Sendai virus (SeV) vector installed with OCT4, SOX2, KLF4 and c-Myc (Nishimura et al., 2011). The three hiPSC clones that were derived from patient HCHWA-D fibroblasts were named LUMCi005-A, LUMCi005-B and LUMCi005-C (Table 1). All hiPSC clones showed typical hiPSC-like morphology with small and tightly packed cells, a high nucleus to cytoplasm ratio, and well defined nucleoli (Fig. 1A). Moreover, all clones were SeV negative at passage 5 as confirmed by Q-RT-PCR (Supplementary Fig. S1) and express the pluripotency markers OCT3/4, NANOG and SSEA-4 (Fig. 1B). In accordance with the immunofluorescence results, the generated hiPSCs showed upregulated expression of the pluripotency genes OCT4, NANOG and SOX2 compared to fibroblasts (Fig. 1C). A routine Global Screening Array (GSA) was performed to check for copy number variants (CNV) or allelic changes and to compare the identities of the fibroblasts and derived hiPSCs. Although the resolution of the GSA array is higher compared to conventional karyotyping, the GSA assay, however, cannot detect balanced translocations. Using a report resolution of ~50 kb, no chromosomal abnormalities were observed (Fig. 1D). Furthermore, the newly derived hiPSCs were identical to the patient-derived fibroblasts (Supplementary Fig. S1). The Dutch mutation was confirmed in all three clones by Sanger sequencing (Fig. 1E). In addition, all clones showed spontaneous differentiation *in vitro* into the three germ layers, as confirmed by immunofluorescent stainings for the endodermal marker α-fetoprotein (AFP), the mesodermal marker PECAM-1 (CD31), and the ectodermal marker β3-tubulin (TUBB3) (Fig. 1F). All hiPSCs were tested for the Apolipoprotein E (ApoE) genotype using the melting curve assay. Apolipoprotein E is a protein with multiple roles in lipid metabolism, neurobiology and neurodegenerative diseases that have been implicated with amyloidopathies. The ApoE2 genotype was shown to be protective, ApoE3 to be neutral and the ApoE4 genotype to be detrimental (Huang et al., 2014). All lines contained an ApoE3/4 genotype. Finally all cell lines were negative in regular tests for mycoplasma (Supplementary Fig. S1). All data mentioned are presented in Table 2.

3. Materials and methods

3.1. Ethical statement

This study was approved by the LUMC Medical Ethical Committee (MEC) and informed consent was obtained from the HCHWA-D patient (NL45478.058.13/P13.080).

3.2. Generation of hiPSCs

A skin biopsy was obtained from 56-year old symptomatic HCHWA-D patient. Following dissection, fibroblasts were cultured in minimum essential medium supplemented with 15% FBS, 2 mM GlutaMAX and 1% penicillin-streptomycin (all ThermoFisher) at 37 °C and 5% CO_2. Fibroblasts were expanded up to passage three and then frozen in liquid nitrogen for future use. For reprogramming 1×10^5 fibroblasts were transduced with 7.5 MOI SeVdp (KOSM) 302 L and seeded on irradiated mouse embryonic fibroblasts (MEFs) in fibroblast media 24 h later. The following day, cells were cultured in DMEM/F12 Glutamax medium supplemented with 20% KnockOut Serum Replacement (KOSR), Non-Essential Amino Acids (NEAA), 2-mercaptoethanol, penicillin-streptomycin (all Gibco) and 10 ng/ml bFGF (Peprotech) until the formation of hiPSC colonies, around 3 weeks later. hiPSC colonies were picked manually and expanded on Vitronectin XF coated plates in TESR-E8 media according to manufacturer's instructions (STEMCELL Technologies). hiPSCs were passaged once per week and within the first five passages the splitting ratio went from 1:2 to 1:10. The cells were dissociated using the gentle cell dissociation reagent (GCDR) (STEMCELL Technologies).

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse IgG2b anti-Oct-3/4	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
	Mouse IgG1 anti-Nanog	1:150	Santa Cruz Biotechnology Cat# sc-293,121, RRID:AB_2665475
	Mouse IgG3 anti-SSEA-4	1:30	BioLegend Cat# 330402, RRID:AB_1089208
Differentiation markers	Mouse IgG2a anti-β3-tubulin	1:4000	Covance Research Products Inc. Cat# MMS-435P, RRID:AB_2313773
	Mouse anti-CD31	1:100	Dako Cat# M0823, RRID:AB_2114471
	Rabbit IgG anti α-fetoprotein (AFP)	1:25	Quartett, Cat# 2011200530, RRID:AB_2716839
Secondary antibodies	Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:250	Thermo Fisher Scientific Cat# A-21242, RRID:AB_2535811
	Goat anti-Mouse IgG3 Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:250	Thermo Fisher Scientific Cat# A-21151, RRID:AB_2535784
	Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:250	Thermo Fisher Scientific Cat# A-21124, RRID:AB_2535766
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:500	Thermo Fisher Scientific Cat# A-11031, RRID:AB_144696
	Donkey anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-21206, RRID:AB_2535792

Primers		
	Target	Forward/Reverse primer (5′-3′)
SeV based vectors (qPCR)	SeV	GCAGCTCTAACGTTGTCAAA/CCTGGAGCAAATTCACCATGA
	GAPDH	TCCTCTGACTTCAACAGCGA/GGGTCTTACTCCTTGGAGGC
Pluripotency Markers (qPCR)	OCT4	TGTACTCCTCGGTCCCTTTC/TCCAGGTTTTCTTTCCTAGC
	NANOG	CAGTCTGGACACTGGCTGAA/CTCGCTGATTAGGCTCCAAC
	SOX2	GCTAGTCTCCAAGCGACGAA/GCAAGAAGCCTCTCCTTGAA
House-Keeping Genes (qPCR)	GAPDH	AGCCACATCGCTCAGACACC/GTACTCAGCGGCCAGCATCG
Genotyping by Sanger sequencing	APP	CAAATGTCCCCTGCATTTAAG/CAAGCATCATGGAAGCACA product size: 382 bp
ApoE genotyping	ApoE	TAAGCTTGGCACGGCTGTCCAAGGA/ ACAGAATTCGCCCCGGCCTGGTACAC Product size: 244 bp

3.3. Spontaneous in vitro differentiation of hiPSCs

Undifferentiated hiPSC colonies were scraped after incubation with Gentle Cell Dissociation Reagent (STEMCELL Technologies) and large pieces were plated on Vitronectin XF coated glass coverslips in TESR-E8 media consistent with manufacturer's protocol (STEMCELL Technologies). After 1 day cells were cultured in DMEM/F12 with 20% FBS for three weeks with media changes every other day.

3.4. Immunofluorescent staining

For immunofluorescent staining hiPSCs were first fixed in 2% paraformaldehyde for 30 min at RT, permeabilized with 0.1% Triton X-100 and blocked with 4% normal swine serum (NSS, DAKO) for 1 h at RT. Then cells were incubated with primary antibodies in 4% NSS, O/N at 4 °C followed by probing with secondary fluorescent dye labelled antibodies for 1 h at RT. DAPI was used as nuclear counter staining. Antibodies are listed in Table 3. Images were generated using a Leica TCS SP8.

3.5. RNA isolation and RT-qPCR

The ReliaPrep™ Miniprep System (Promega) was used for RNA isolation according to manufacturer's instructions. 500 ng of RNA per reaction were used for cDNA synthesis using the transcriptor first strand cDNA synthesis kit (Roche). Cycling parameters of the qPCR included an initial denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s and extension at 72 °C for 20 s. The RT-qPCR reaction was run on a LightCycler 480 Real-Time PCR System (Roche) with SensiMix SYBR Hi-ROX Kit (Bioline). CT-values were normalized to GAPDH using the ΔΔCT-method. Primer sequences are listed in Table 3.

3.6. Genomic DNA isolation

The Wizard Genomic DNA purification Kit (Promega) was used for genomic DNA isolation according to the manufacturer's instructions.

3.7. Sanger sequencing

To confirm the presence of the Dutch mutation in the APP gene, 100 ng of gDNA from all 3 clones was used for PCR (T100 Thermal Cycler, Bio-Rad). Cycle parameters were an initial denaturation at 95 °C, followed by 39 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s and elongation at 72 °C for 30 s. Final elongation step at 72 °C for 7 min. Primer sequences are listed in Table 3. PCR samples were submitted for Sanger sequencing and sequences were checked with the SnapGene software.

3.8. ApoE genotyping

To check the ApoE variant that is present in the hiPSC lines, 100 ng of gDNA from all 3 clones was used for RT-PCR (DNA engine, Bio-Rad). Cycle parameters were initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 64 °C for 30 s and extension at 72 °C for 30 s. Final elongation step at 72 °C for 5 min. Then PCR samples were inserted in the Lightscanner 96 Hi-res Melting System (Idaho Technologies) for the melting curve assay, with starting temperature at 75 °C and ending temperature at 98 °C. Results were analyzed with the Lightscanner software.

3.9. Mycoplasma detection

The MycoAlert™ Mycoplasma Detection Kit (Lonza) Tests were used for testing mycoplasma presence in the cell cultures according to the manufacturer's instructions.

3.10. Global screening array

The Global Screening Array (GSA) (Illumina) was used according to standard procedures, followed by an analysis in GenomeStudio software (Illumina) using the GSA manifest files. GenomeStudio final reports were used to analyze and visualize in Nexus Discovery (BioDiscovery El Segundo). A report resolution of ~50 kb was used to analyze the data for chromosomal aberrations. To compare the patient fibroblasts with the generated hiPSCs the array final reports were selected as input for an R script. The files were transformed into smaller tables based on their SNP ID. Using statistics we determined whether the allelic calls matched, mismatched or failed.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.101359.

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References

Bornebroek, M., Haan, J., Maat-Schieman, M.L.C., Van Duinen, S.G., Roos, R.A.C., 1996. Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D): I - a review of clinical, radiologic and genetic aspects. Brain Pathol. 6, 111.

Huang, Y., Mahley, R.W., Apolipoprotein, E., 2014. Structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiol. Dis. 72, 3.

Kamp, J.A., Moursel, L.G., Haan, J., Terwindt, G.M., Lesnik Oberstein, S.A.M.J., Van Duinen, S.G., et al., 2014. Amyloid β in hereditary cerebral hemorrhage with amyloidosis-Dutch type. Rev. Neurosci. 25, 641.

Nishimura, K., Sano, M., Ohtaka, M., Furuta, B., Umemura, Y., Nakajima, Y., et al., 2011. Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming. J. Biol. Chem. 286, 4760.